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Plant Chemistry, Soil Microbiology and
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CONTENTS: TABLE DES MATIÈRES: INHALT:

<i>J. Drobnik</i> , Primary oxidation of organic matter in the soil. I. The form of respiration curves with glucose as the substrate	199
<i>J. Drobnik</i> , Primary oxidation of organic matter in the soil. II. Influence of various kinds of preincubations	212
<i>P. Riepma Kzn.</i> , Interrelationships between methods of spray application, retention, and weather conditions on the herbicidal efficiency of 2,4- dinitro-ortho-cresol	223
<i>J. Bruinsma</i> , The action of 4,6-dinitro-o-cresol (DNOC) in soil. I. The emergence from cress seed in DNOC-treated soil mixtures.	249
<i>R. K. Kirsch, M. E. Harward, and R. G. Petersen</i> , Interrelationships among iron, manganese, and molybdenum in the growth and nutrition of tomatoes grown in culture solution	259
<i>F. Jacquin et F. Mangenot</i> , Populations microbiennes des bois. IV. Humification de copeaux dans la nature	276
<i>J. Hagin</i> , On the shape of the yield curve.	285

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PRIMARY OXIDATION OF ORGANIC MATTER IN THE SOIL

I. THE FORM OF RESPIRATION CURVES WITH GLUCOSE AS THE SUBSTRATE

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INTRODUCTION

In our previous work ⁹ we discussed the question of the humification and mineralization of organic matter added to the soil and we studied the factors controlling the proportion of the added carbon which was humified and mineralized. In macrorespirometrical experiments we demonstrated that respiration curves could give valuable information about the mineralization processes and about other problems of soil metabolism. For this reason, the study of such curves has been the objective of our recent investigations, where the first step as described in this paper, was the estimation of the most convenient method for their quantitative evaluation.

In the study of soil metabolism three types of experimental system can be used: a closed, an open, or an extended system. An open system is realized in the method of continuous perfusion of sample with a solution containing the substrate ²². The percolation method introduced by Lees and Quastel ²¹, Greenwood and Lees ¹⁵, Wheeler and Yemm ²⁷, and others represents an extended system. It differs from the first method by the fact that no fresh substrate is added during the course of experiment and the metabolites are not removed (except when sampling the solution). As in the case of the open system the micro-organisms are allowed to react with a greater volume of solution of substrate than the corresponding water-holding capacity of the soil sample and

the movement of the solution is similar to that of the open system. Consequently, all changes are more gradual than in a closed system.

In our experiments⁹ a closed system was used similar to that employed by Rovira^{24 25}, Katznelson and Stevenson¹⁸, Stevenson and Katznelson²⁶, and others. In this case the microbial association reacts fully with the substrate. When the experiment is continued for a sufficiently long period the end-products, *i.e.* mineral compounds and humus, will be formed.

One per cent of substrate used in long-term experiments with a macrorespirometer may cause great changes in the composition of the soil microflora¹³. Since we are interested in the activity of a natural association, we therefore turned in this study to the Warburg technique and diminished the substrate concentrations down to 5–10 μ M per 4-gram sample *i.e.* to 0.045% of glucose, and reduced the period of experiment to one to three days. As Katznelson and Stevenson¹⁸ have stated no significant changes in numbers of organisms occur in these conditions.

EXPERIMENTAL METHODS

1. Soils

The soil C is the same meadow sandy-loam as used previously^{8 9}. The soil L is a partially decalcificated tschernozem under corn. The characteristic of horizons are given later. Samples were collected in the autumn 1957.

The samples were sieved through a 2-mm sieve. In the control experiments we found that drying changed the shape of respiration curves and stimulated the endogenous respiration^{1 24}. On the other hand the dry soil maintained its characteristic course of respiration during at least 24 days which was the time necessary for experiments with soil C. For these reasons we dried the soil C to obtain comparable results from the whole series of experiments with it but did not dry the soil L in order to avoid the modification of respiration curves and finished all experiments with it in three days.

2. Warburg technique

To a 4-gram sample in a conventional Warburg vessel we added a suitable quantity of water (or glucose solution) so as not to log the sample. In preliminary experiments we found that the amount of water did not affect the results provided the sample was sufficiently moistened but not waterlogged (see also Ref. ¹¹). We added the solution of substrate directly to the sample for it was not possible to decant the substrate quantitatively from

the side arm. The respiration during the time necessary for equilibration (10 to 30 min) was calculated. In the centre well we placed 0.2 ml of 20% potassium hydroxide. The temperature was 28°C. Manometers and vessels were not shaken.

3. *Estimation of enzyme activity*

Activities of saccharase and asparaginase were measured as described previously^{7 10}. The results are given in ml of 0.05 *N* sodium thiosulphate for 1 ml of supernatant (a 5-g sample was incubated for 24 hours with toluene, 2.5 ml of 10% sucrose, and 5 ml water at 42°C, then made up to 25 ml; the reducing substances were estimated in 1 to 5-ml portions), or in ppm of ammonium split off from asparagin (a 5-g sample was incubated for 48 hours with toluene and 5 ml of 0.45 *M* asparagin solution at 42°C).

4. *Chemical analyses*

Glucose was estimated according to the method described by Frank and Kirberger¹². Immediately after removing the Warburg vessel from the waterbath we added dilute sulphuric acid to make up the volume of the solution to 8 ml and the pH to 1.5–2. After stirring and filtering we took 1 ml of filtrate, added 1.9 ml of sodium sulphate–copper sulphate solution and 0.1 ml of sodium tungstate solution. After centrifugation glucose was estimated colorimetrically using a Spekker absorptiometer with red filters (No 8) and 1-cm cells. The concentration of glucose was directly proportional to absorptiometer readings. Other chemical analyses were carried out by the conventional methods in use in our laboratory⁹.

EXPERIMENTAL RESULTS

1. *Typical respiration curve with 10 μ M of glucose as the substrate*

In this experiment, we used soil C. The results are given in Fig. 1. The points are the mean values of four to six replicate samples, the vertical lines show the range of variation. The control values are subtracted.

The cumulative curve is, as usual, of sigmoid shape. The derived curve representing the changes of respiration rate has a standard form. In the first four hours, it rises very slowly from 8 μ l to 12 μ l per hour. Between the fourth and sixth hours the respiration rate reaches its maximum. From this point it drops very rapidly at first but from the twelfth hour it diminishes more slowly over a period of seven hours. Beginning at the twenty-first hour the curve starts to follow the exponential course which is typical of "biological equilibrium" as postulated by Chase and Gray^{3 4} and confirmed by our experiments^{8 9}.

It can be seen from the amount of glucose remaining that the

rapid fall of respiration rate in the tenth hour is caused by complete exhaustion of substrate.

According to these results we may divide the process of glucose mineralization into two main parts: *primary* and *secondary oxidation*. The period when substrate is still present in the sample (part I in Fig. 1) we call *primary oxidation*. From the shape of

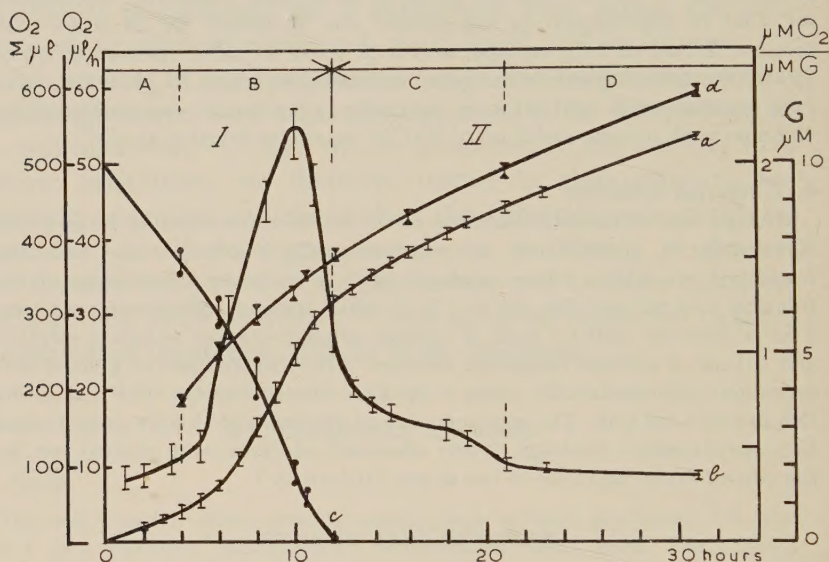


Fig. 1. The oxidation of $10 \mu\text{M}$ of glucose in 4-g samples of soil C. The variation between six of more replicate experiments are shown.

- a – cumulative oxygen consumption in μl per 4-g sample
- b – respiration rate in μl per 4-g sample per hour
- c – glucose content in μM in 4-g sample
- d – oxygen absorbed: glucose consumed ratio in $\mu\text{M}/\mu\text{M}$

the respiration curve we can see that this stage consists of two subdivisions. The course of respiration up to the fourth hour indicates that glucose is predominantly oxidized by enzymatic system originally present in the soil sample in micro-organisms and, may be, occurring freely in the soil. Therefore this phase is called an *oxidative component* (section A). Since the logarithmical increase is apparently due to synthesis of enzymes, *i.e.* is connected with building up of cellular materials, we characterize the second phase involved in this increase as an *assimilative component* (section B).

The *secondary oxidation* (period II) is not uniform, too. There is an *intermediate stage* at first in which the decrease of respiration is not yet exponential (section C). The exponential decrease characterizes the next stage called *residual oxidation* (section D).

In the first steps of primary oxidation the ratio of oxygen absorbed to glucose consumed (O_2/G) is low. At the end of primary oxydation the rate reaches one and a half molecules of oxygen per molecule of glucose increasing to two molecules at the end of the intermediate stage. These values are in good agreement with our previous findings^{8 9}. To verify the general meaning of these values we performed the following experiments.

2. The effect of glucose concentration on the primary oxidation

Because the O_2/G ratio at the end of primary oxidation is very important from the point of view of the equilibrium between mineralization and humification we wish to know in what manner it is affected by glucose concentration. The results of experiments on this point are given in Fig. 2 and Table 1. It is apparent from

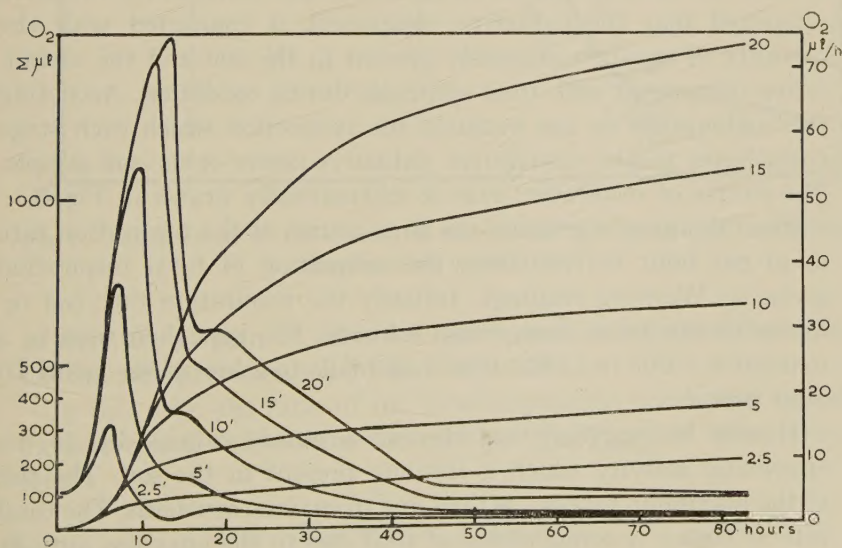


Fig. 2. The oxidation of glucose in various concentrations in 4-g samples of soil C.

2.5 - 20 cumulative oxygen consumption during oxidation of 2.5 to 20 μM of glucose in 4-g samples.

2.5' - 20' respiration rates.

TABLE 1

The ratio of oxygen absorbed to glucose consumed in the primary oxidation of various concentrations of glucose in the soil sample					
Glucose concentration μM . . .	2.5	5.0	10.0	15.0	20.0
The end of prim. ox., hours . .	11.5	11.5	12.5	16.0	16.0
O ₂ absorbed, μl	84.4	190.7	325.9	541.0	706.4
μM O ₂ : μM glucose consumed .	1.51	1.70	1.46	1.55	1.57
The end of interm. stage, hours .	—	21	29	39.5	39.5
O ₂ absorbed, μl	—	255	532	904	1235
μM O ₂ : μM glucose consumed .	—	2.28	2.38	2.69	2.76

the data obtained that the O₂/G ratio remains constant at the end of primary oxidation irrespective of glucose supply. On the contrary, it rises at the end of the intermediate stage of the secondary oxidation with an increasing glucose concentration.

3. *The estimation of the relationship between oxidative and assimilative components and the influence of the biological potency of soil*

The primary oxidation consists of the oxidative and the assimilative stages. There is a possibility that the relative magnitudes of both can influence the ratio O₂/G of primary oxidation. We have suggested that the oxidative component is connected with the quantity of enzymes originally present in the soil and the assimilative component with their synthesis during oxidation. Accepting this assumption we can evaluate the proportion which each stage contributes to the cumulative oxidative power of the soil sample. The course of respiration rate is schematically drawn in Fig. 3.

The full curve represents the time course of the respiration rate in μl per hour derived from the estimation of total respiration given by Warburg readings. Initially the respiration rate (s_0) remains steady for a time period b hours. The rate then rises to a maximum value (σ_c) after time c and falls to a low value (point D) after time d .

It may be supposed that the initial steady rate is due to the enzymatic activity which is initially present in the soil. The rise to the maximum rate (σ_c) is then due to enzyme synthesis. The total rate at time c is compounded of that due to the enzymes initially present in the soil and that due to the newly synthesized enzymes. By extrapolation of the initial rate to point C' on Fig. 3 these two parts, the oxidative and the assimilative components of the respiration, can be evaluated up to time c .

If it be assumed that during the subsequent decrease of respiratory rate (which is due – as we have seen – to glucose deficiency) there is no change in enzyme concentration and that the two components, oxidative and assimilative, are related in magnitude

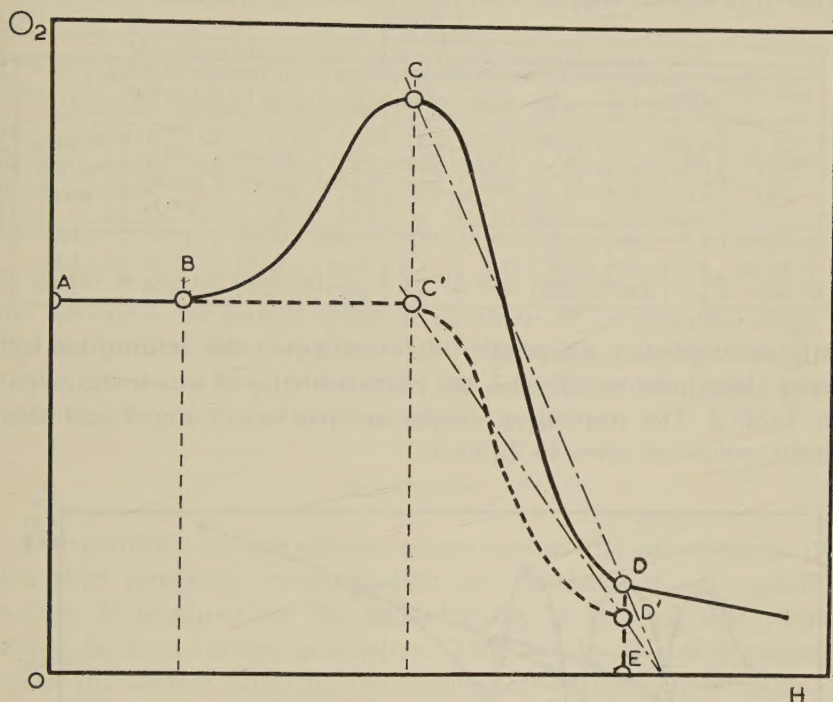


Fig. 3. The schema of the course of respiration during glucose oxidation.
Explanation in the text.

as they are at point C, then we can further extrapolate the curve for the oxidative component from C' to D'.

In this way, the ratio of the total respiration occasioned by the oxidative and assimilative components Σ_{ox}/Σ_{ass} can be obtained for:

$$\Sigma_{ox} = s_0 \cdot c + \Sigma_p \cdot \frac{s_0}{\sigma_c}$$

where Σ_p = total respiration in time D–C.

Then the relationship of the oxidative and assimilative components can be evaluated as Σ_{ox}/Σ_{ass} .

The size of the oxidative component is apparently determined by the amount of glucose-oxidizing enzymes present in the soil. The amount of enzymes in soil is roughly correlated with the biological potency of soil¹⁷ and diminishes in the deep horizons⁶. Therefore we may suppose that the oxidative component diminishes

TABLE 2

Some chemical and biological characteristics of the horizons of soil L (Detailed description of humus fractions is given by Najmr ²³)									
Horizon cm	pH	C %	N %	NH ₃ ppm	NO ₃ ppm	Sac- charase	Aspara- ginase	Resp. μl O ₂ per 4 g per h.	Bacteria mil. per gram
A' 0-40	7.7	1.68	0.157	5.6	17.0	1.40	81.00	13.5	11.65
A" 40-65	8.0	1.30	0.128	4.9	1.8	0.60	19.70	5.25	5.80
C 65	8.1	0.30	0.020	0	6.7	0.18	21.00	6.50	1.95

with the depth in a soil profile. We investigated this assumption by using three horizons of soil L the characteristics of which are given in Table 2. The respiration curves are plotted in Fig. 4 and the mean values are given in Table 3.

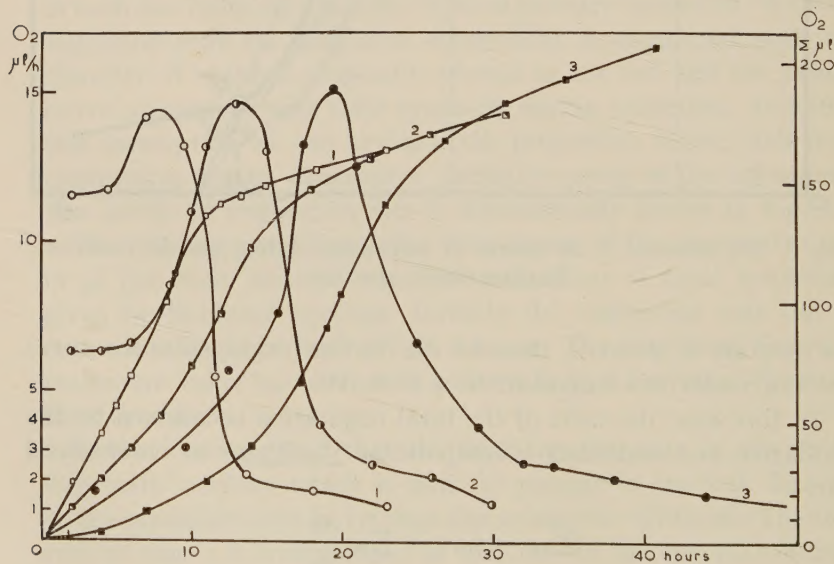


Fig. 4. The oxidation of 5 μ M of glucose in three horizons of soil L.
1, 2, 3 – cumulative oxygen consumption by samples of A', A" and C horizons
1', 2', 3' – respiration rates

The results confirm our assumption. The assimilative stage begins later in deeper horizons (because the number of micro-organisms is lower). The proportion of the oxidative to the assimilative components (measured as the amount of oxygen consumed by each

TABLE 3

The characteristic points of respiration curves of soils C and L										
Soil	Conc. of glucose	<i>b</i> hours	<i>c</i> hours	<i>d</i> hours	<i>s</i> ₀ μl/h	<i>σ</i> _c μl/h	<i>Σ</i> _p μl	<i>Σ</i> _{ox} μl	<i>Σ</i> _{ass} μl	<i>Σ</i> _{ox} / <i>Σ</i> _{ass} μl : μl
C	10 μ <i>M</i>	4.5	10.0	12.0	10.0	55.0	320.0	113.5	206.5	0.55
L-A'	5 μ <i>M</i>	4.5	8.0	13.5	11.5	14.4	114.3	127.0	17.3	7.35
L-A''	5 μ <i>M</i>	6.0	13.5	19.0	6.3	15.0	152.3	100.0	52.3	1.91
L-C	5 μ <i>M</i>	0	19.5	32.0	0	15.1	186.5	0	186.5	0

of them) is in the horizon A' 7.35, in the horizon A'' 1.91, and in the horizon C we cannot speak at all about of an oxidative stage. The O₂/G ratio rises with the depth. It seems possibly a result of the increasing preponderance of the assimilative component but other factors may have to be considered here.

DISCUSSION

The turnover of the organic substances in the soil consists of two chief processes: mineralization and synthesis of soil organic matter. It is important for good fertility of the soil that they should be in a certain proportion. That is why in our previous works the factors affecting the mineralization were investigated. Being in fact an oxidation, the mineralization can be followed by measuring the oxygen consumption. Therefore the detailed analysis of respiration curves can be useful for further study.

The whole process of mineralization of simple compounds can be divided, according to the results of our own and other authors' experiments, into several stages. The first stage is a direct transformation of the compound used as substrate. We call it the *primary oxidation*. It is characterized by an increase of oxygen consumption caused directly by the addition of substrate. As a simultaneous disappearance of the added compound is observed we assume that the additional oxygen absorbed (as compared with the control) is consumed for its oxidation. It will be shown later that this suggestion is not entirely indisputable. From the point of view of the synthesis of soil organic matter it is evident that the part of the

added substance which is oxidized in the primary process of oxidation (mineralized) can no longer serve as a substrate for the synthesis of the soil organic matter. Also the remaining portion does not persist in its original form. It is partly converted into various metabolites, partly into components of micro-organism cells. According to their nature the metabolites can be further oxidized. As long as this happens while the original substrate is not yet converted their turnover cannot be studied. After its oxidation, however, the oxidation of metabolites will manifest itself by the oxygen consumption rate not dropping exponentially immediately after the exhaustion of the substrate. It remains, however, for some time at a higher level than could be deduced from theoretical curve. This delay is called the *intermediate stage* of the *secondary oxidation*. A portion of the substrate is converted into the cellular material of the micro-organisms, partly by increasing their number, partly by forming reserve compounds and new enzymatic systems. This results, necessarily, in an increase of the endogenous respiration. This increase of endogenous respiration together with the oxidation of difficultly decomposable metabolites (*e.g.* polysaccharides) and of dead microbial cells will manifest itself by the consumption of oxygen the exponential course of which is characteristic of the biological equilibrium of the soil but at a higher level than in the control soil. This stage which lasts usually for a very long time is called the *residual oxidation*.

A sudden increase in oxygen uptake can be evoked not only by the addition of an oxidizable substrate but also by other treatments which interfere with the biological equilibrium of soil (*e.g.* sterilization by heat ¹⁹ ³, chemicals, by an increase of pH ²⁴, by the addition of a source of nitrogen into a deficient soil ⁸, *etc.*). In this case, the soil organic matter is oxidized and this oxidation is called *reactive* because there is a reaction of the soil microbial association to the destruction of the biological equilibrium.

There is a possibility that the added substrate possesses also a similar unspecific effect, and, at the same time, evokes the reactive oxidation of soil organic matter. It follows from the work of Hallam and Bartholomew ¹⁶, Broadbent ², and others that the addition of decomposable matter stimulates the decay of the soil organic matter. Further, it is evident that the micro-organisms need for their synthetic activity sources of other nutrients, es-

pecially nitrogen and phosphorus as well as a source of carbon and energy supply. When these nutrients are not available in a sufficient quantity in a mineral form, the microbes must draw them by decomposing the soil organic matter, as Gray and Taylor¹³ have suggested in the case of cellulose. It is very probable that in the stage of primary oxidation the amount of oxygen consumed equals the sum of the oxygen uptake in the primary oxidation of the added matter, and the oxygen absorbed in the reactive oxidation of the soil organic matter. It is impossible, at present, to separate these two portions by our methods. At present we use the term "primary oxidation" for the sum of both portions.

According to our results it is further evident that in the primary oxidation itself it is possible to distinguish two stages: the *oxidative* and the *assimilative* ones. It follows from the data of Stevenson and Katznelson²⁶ and from works on soil enzymes that the oxidative component is dependent on the biological potency of the soil. We assume the enzymes directly oxidizing glucose such as glucose-oxydase play a dominant role in that process. The intermediary stage of the secondary oxidation can, in some cases, be observed distinctly (Fig. 2), at other times it cannot (Fig. 4). That can be explained by suggestion that in the second case the intermediary stage fuses with the primary oxidation. Regarding these results we wish, in our studies on mineralization, to deal, in the first place, with the primary oxidation which is the foremost determining factor influencing the proportion of an added organic substance which remains available for the synthesis of the soil organic matter. As follows from previous experiments, and due to its exponential course, the residual oxidation has, for various substrates and conditions, an uniform character and differs only by the magnitudes of the constants F' and m^{5348} .

SUMMARY

The course of respiration rate during glucose decomposition in a soil sample was studied. The whole process of oxidation (mineralization) is divided into two principal parts: the primary oxidation which occurs while the substrate is still present and the secondary oxidation after the disappearance of the substrate. The primary oxidation brought about by two different components: the oxidative component represents the sum of enzyme activity present originally in the soil sample, and the assimilative

component is built up during the process by synthesis of microbial enzymes. The size of the oxidative component is correlated with the biological potency of soil. Sometimes, an intermediary stage can be observed at the beginning of the secondary oxidation. A method is given for enumeration of the proportions of the two components involved in primary oxidation. The biochemical and pedological meaning of these data is discussed.

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REFERENCES

- 1 Birch, H. F., The effect of soil drying on humus decomposition and nitrogen availability. *Plant and Soil* **10**, 9-31 (1958).
- 2 Broadbent, F. E., Nitrogen release and carbon loss from soil organic matter during decomposition of added plant residues. *Soil Sci. Soc. Am. Proc.* **12**, 246-249 (1947).
- 3 Chase, F. E. and Gray, P. H. H., Use of the Warburg respirometer to study microbial activity in soil. *Nature* **171**, 481 (1953).
- 4 Chase, F. E. and Gray, P. H. H., Application of the Warburg respirometer in studying respiratory activity in soil. *Can. J. Microbiol.* **3**, 335-349 (1957).
- 5 Corbet, S. A., Studies on tropical soil microbiology: I. The evolution of carbon dioxide from the soil and the bacterial growth curve. *Soil Sci.* **37**, 109-115 (1934).
- 6 Drobnič, J., Štěpení škrobu enzymatickým komplexem půd. *Českoslov. Biol.* **4**, 19-29 (1955).
- 7 Drobnič, J., Štěpení asparaginu enzymatickým komplexem půd. *Českoslov. Mikrobiol.* **1**, 47 (1956).
- 8 Drobnič, J., Biological transformations of organic substances in the soil, (in Russian with an English summary). *Pedology (USSR)* **1957** (12), 62-71.
- 9 Drobnič, J., A respirometric study of glucose metabolism in soil samples. *Folia Biol. (Prague)* **4**, 137-146 (1958).
- 10 Drobnič, J. and Seifert, J., Vztah enzymatické inverse sacharosy v půdě k některým půdně mikrobiologickým testům. *Českoslov. Biol.* **4**, 30-35 (1955).
- 11 Ellinger, G. and Quastel, J. H., Preliminary experiments in the study of the respiratory activity of microorganisms suspended in thin films of fluid adhering to solid surfaces. *Biochem. J.* **42**, 214-218 (1948).
- 12 Frank, H. and Kirberger, E., Eine kolorimetrische Methode zur Bestimmung der „wahren Glucose“ und Galactose in 0,05 ml Blut. *Biochem. Z.* **320**, 359-367 (1950).
- 13 Gray, P. H. H. and Taylor, C. B., The aerobic decomposition of glucose in podsol soils. *Can. J. Research C* **17**, 109-124 (1939).
- 14 Gray, P. H. H. and Taylor, C. B., Microbiological studies of Appalachian podsol soils. IV. The decomposition of glucose in soil previously treated with amendments. *Can. J. Research C* **17**, 147-153 (1939).
- 15 Greenwood, D. J. and Lees, H., Studies on the decomposition of amino acids in soils. I. A preliminary survey of techniques. *Plant and Soil* **7**, 253-267 (1956).
- 16 Hallam, M. J. and Bartholomew, W. V., Influence of rate of plant residue addition in accelerating the decomposition of soil organic matter. *Soil Sci. Soc. Am. Proc.* **17**, 365-368 (1953).
- 17 Hofmann, E., Enzymreaktionen und ihre Bedeutung für die Bestimmung der Bodenfruchtbarkeit. *Z. Pflanzenernähr. Düng. u. Bodenk.* **56**, 68-72 (1952).
- 18 Katznelson, H. and Stevenson, I. L., Observations on the metabolic activity of the soil microflora. *Can. J. Microbiol.* **2**, 611-622 (1956).

- 19 Koepf, H., Untersuchungen über die biologische Aktivität des Bodens. Teil I. Atmungskurven des Bodens und Fermentaktivität unter dem Einfluss von Düngung und Pflanzenwachstum. *Z. Acker- u. Pflanzenb.* **98**, 289–312 (1954).
- 20 Lees, H., The soil percolation technique. *Plant and Soil* **1**, 221 (1949).
- 21 Lees, H. and Quastel, J. H., A new technique for the study of soil sterilization. *Chem. & Ind. London* **26**, 238–239 (1944).
- 22 Macura, J. and Málek, I., Continuous-flow method for the study of microbiological processes in soil sample. *Nature* **182**, 1796–1797 (1958).
- 23 Najmr, S., Humus ve výrobně důležitých půdních typech. St. zem. nakl. Praha (1957).
- 24 Rovira, A. D., Use of the Warburg apparatus in soil metabolism studies. *Nature* **172**, 29 (1953).
- 25 Rovira, A. D., Plant root excretions in relation to the rhizosphere effect. III. The effect of root exudate on the numbers and activity of micro-organisms in soil. *Plant and Soil* **7**, 209–217 (1956).
- 26 Stevenson, I. L. and Katznelson, H., The oxydation of ethanol and acetate in soils. *Can. J. Microbiol.* **4**, 73–79 (1958).
- 27 Wheeler, B. E. J. and Yemm, E. W., The conversion of amino acids in soils. I. Amino-acid breakdown and nitrification in cultivated and natural soil. *Plant and Soil* **10**, 49–77 (1958).

PRIMARY OXIDATION OF ORGANIC MATTER IN THE SOIL

II. INFLUENCE OF VARIOUS KINDS OF PREINCUBATIONS

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INTRODUCTION

In a previous work ³ we described the typical course of respiration during oxidation of glucose in a soil sample. We divided the whole process of oxidation (mineralization) into two principal parts – the primary, and the secondary oxidation. The results show that the primary oxidation is composed of two components, the oxidative and assimilative components, whose magnitudes are related to the biological potency of soil. These findings are in good agreement with the opinions of Birch ¹ and Corbet ² that the course of the respiration curves of a soil sample is determined by the development of its microbial population.

We made some further experiments to elucidate the effect of the biological factor on the course of respiration. The biological component of the soil sample was influenced by various conditions of preincubation. The results of these experiments are presented in this paper.

METHODS

The same meadow sandy-loam (soil C) was used as in the previous work ³ but the samples were collected in spring. After sampling, the damp (12.3% moisture) soil was sieved and portions of 500 g were placed in wide-necked five-liter flasks and covered with filter paper. The incubation was carried out at room temperature. Every day the samples were mixed and, from time to time, the moisture loss was adjusted after weighing. The fluctuations were less than $\pm 1\%$. A solution of the substrate or water was added by a sprayer: the sample was spread in a thin layer on a PVC foil and a small

portion of solution was sprayed on it. Then, the sample was thoroughly mixed and spraying was repeated until the required volume had been added. No substrate was added during the last four days of preincubation. Twenty four hours before the end large aggregates of soil were disrupted, the sample sieved again, and placed into the thermobox at a temperature of 28°C.

The preincubation was carried out with following variations:

I. The soil was stored at its natural moisture content for 93 days. Water only was added when necessary.

II. Mineral elements were added at the beginning (0.66 g diammonium phosphate, *i.e.* 0.028% N), then the sample was incubated as in I.

III. As in II, except that 24 hours before the end the sample was air dried.

IV. As in II, except that 24 days before the end a 50-g portion was placed in a 500-ml flask which was then closed by a glass stopper and paraffin and placed into the thermobox. The gas given off was liberated from time to time by heating the paraffin. The time interval between the opening of the flask and the beginning of the measurement of the respiration was 4.5 hours.

V. At the beginning diammonium phosphate was added as in II but later the carbon and nitrogen sources (1% of glucose and 0.256% of ammonium carbonate) were added each fourth day. After five additions the reducing substances were not completely destroyed and the amount of inorganic phosphates had decreased. Therefore a one-week break was made during which potassium phosphate was added. Then the additions of glucose and diammonium phosphate were started again but the intervals were prolonged to five or six days. This caused glucose to be completely destroyed. After ten additions diammonium carbonate was omitted (11th addition) and then the portion of glucose was diminished to 0.5% (12th addition) to prevent accumulation of ammonium nitrogen and undestroyed glucose or metabolites. In next eight days no substrate was added and then the respiration was measured.

Warburg techniques: In the conventional Warburg vessels 1.5-g samples (on a dry-weight basis) were placed. In one side arm 1 ml of substrate was pipetted and water up to 2 ml in the second one. At zero time the solutions were thoroughly mixed with the sample to form a suspension. 0.2 ml of 20% potassium hydroxide were placed in the central well. Temperature 28°C. Shaking 100 strokes per minute.

RESULTS AND DISCUSSION

The results of chemical analyses of the samples after preincubation are given in Table 1 and the endogenous respiration in Fig. 1.

1) The respiration of a soil sample immediately after sampling is given in Fig. 2 and Table 2. The oxidative component is relatively high. The respiration rate is not directly proportional to the

TABLE 1

The analyses of samples before respiration experiments						
Kind of preincubation	Moisture %	C %	N %	NH ₃ -N ppm	NO ₃ -N ppm	C/N
0	20	1.40	0.112	5	0	12.3
I	14	1.40	0.113	1	80	12.3
II	16.5	1.44	0.144	75	900	10.0
III	—	1.44	0.144	75	900	10.0
IV	16.5	1.46	0.143	85	0	10.3
V	13.5	1.87	0.229	246	0	8.2

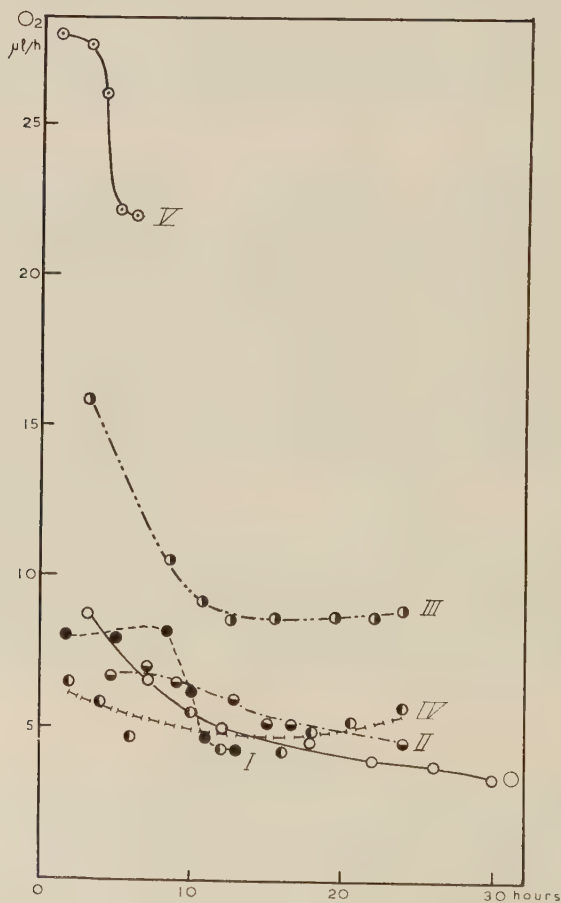


Fig. 1. Respiration rate of soil samples after various preincubations. 0 — without preincubation, I–V variations of preincubation (see text). Without any substrate.

TABLE 2

Primary oxidation of glucose in soil samples after various preincubations (The method of analysis of respiration curves is described in the first article of this series)												
Kind of preincubation	Glucose μM	b hour	c hour	d hour	s_o $\mu l/h$	σ_c $\mu l/h$	Σ_p μl	Σ_{ox} μl	Σ_{ass} μl	Σ_{ox}/Σ_{ass}	$(O_2/G)_p$ (M/M)	$(O_2/G)_{ass}$ (M/M)
0	2.5	—	—	9.0	9.7	—	83.3	83.3	0	∞	1.49	—
	5.0	8.0	10.0	16.0	11.3	14.3	187.9	161.0	27.9	5.76	1.67	5.60
	7.5	8.0	14.0	25.0	11.8	18.4	297.5	225.5	72.0	3.13	1.77	4.00
	10.0	6.0	15.0	28.0	13.4	19.0	418.7	432.0	86.7	3.84	1.87	29.00
I	2.5	0	3.0	5.0	10.0	19.7	71.6	43.8	27.8	1.58	1.28	1.00
	5.0	1.0	6.0	9.5	15.0	19.8	151.0	123.6	27.4	4.50	1.35	0.68
	7.5	1.0	8.5	11.0	15.5	25.2	228.2	169.0	59.2	2.85	1.30	1.08
	10.0	1.0	10.0	15.0	16.5	29.2	330.5	225.8	10.47	2.16	1.48	1.40
II	2.5	—	—	6.5	16.0	—	83.0	83.0	0	∞	1.48	—
	5.0	4.0	8.5	11.0	14.0	27.0	170.0	134.3	35.7	3.77	1.52	1.60
	7.5	2.5	11.0	12.5	14.0	44.0	275.0	162.8	112.2	1.45	1.64	1.87
	10.0	2.5	12.0	14.0	14.0	62.0	396.5	187.0	209.5	0.89	1.77	2.11
III	2.5	0	9.0	11.0	0	15.5	75.7	0	75.7	0	1.35	1.35
	5.0	0	11.5	12.5	0	40.5	157.9	0	157.9	0	1.41	1.41
	7.5	0	13.0	14.5	0	62.5	264.5	0	264.5	0	1.58	1.58
	10.0	0	13.5	15.0	0	83.0	372.1	0	372.1	0	1.66	1.66
IV	2.5	10.0	14.0	15.0	6.0	15.5	128.8	87.0	41.8	2.1	2.30	—
	5.0	10.0	16.0	17.0	7.0	44.0	244.2	115.0	129.2	0.89	2.18	3.70
	7.5	10.0	17.0	18.0	10.0	82.0	373.4	171.6	201.8	0.85	2.22	3.70
	10.0	9.0	17.0	19.0	14.0	128.5	483.7	244.0	239.7	1.02	2.16	3.90
V	2.5	—	—	3.5	91.0	—	98.7	98.7	0	∞	1.77	—
	5.0	0	1.5	3.5	91.0	100.0	215.7	202.0	13.7	16.75	1.92	—
	7.5	0	1.5	3.5	91.0	133.0	320.0	243.0	77.0	3.16	1.91	—
	10.0	0	2.0	5.5	91.0	147.0	497.0	334.0	163.0	2.05	2.22	—

b — beginning of the ass. stage; c — time of the maximum; d — the end of primary oxidation; s_o — resp. rate in the beginning of p.o.; σ_c — maximal resp. rate; Σ_p — consumption of O_2 during p.o.; Σ_{ox} and Σ_{ass} — consumption of O_2 calculated for ox. and ass. components respectively

substrate concentration. The conditions of the fresh sample are apparently not favourable for assimilation as indicated by the long lag of 8 hours, the low maximum rate (the difference between maximal rate σ_c and initial respiration rate s_o is only 6 μl per hour), and the flat maximum given by the highest substrate concentration. Consequently it is concluded that the predominant component is the oxidative one. We suppose the intermediary stage to fuse with the primary oxidation. The following calculation confirms this suggestion:

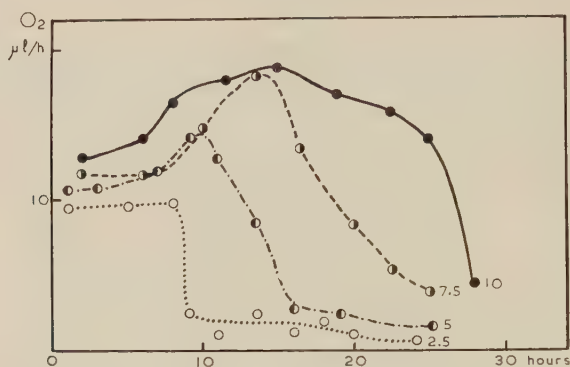


Fig. 2. Respiration rate of soil samples six hours after sampling. The control without substrate is subtracted.

Glucose concentrations:

-○ 2.5 μM per vessel
- 5.0 " " "
- .-.-.-● 7.5 " " "
- 10.0 " " "

Two and a half μM of glucose are consumed before the synthesis of enzymes starts, *i.e.*, only the oxidative component is involved in this process. The ratio of moles of oxygen per mole of glucose added is about 1.5, similar to second variation of preincubation. Assuming this ratio of oxidation by oxidative component $(O_2/G)_{ox}$ being in all concentrations the same, the amount of oxygen per molecule of glucose consumed by assimilative component $(O_2/G)_{ass}$ can be calculated as follows:

$$(O_2/G)_{ass} = \frac{1.5 \cdot \Sigma_{ass}}{1.5 \cdot G - \Sigma_{ox}}$$

where Σ_{ass} and Σ_{ox} are moles of oxygen absorbed by each component and G represents the concentration of glucose.

These calculated values are given in the last column of Table 2. They show that other substances than glucose must be oxidized during assimilative state. The end-products of the oxidative component may be considered.

2) The respiration of the sample stored 93 days in naturally moist state (variation I) is given in Fig. 3 and Table 2. The prolonged preservation in the moist state has resulted in an im-

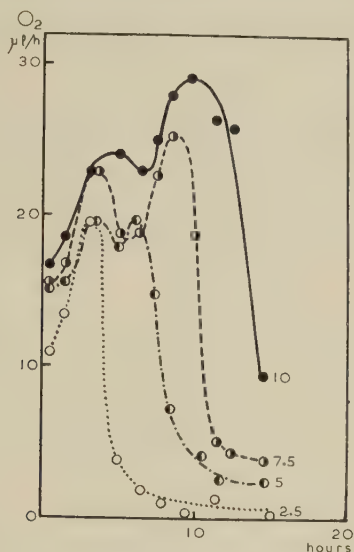


Fig. 3. Respiration rate of soil samples stored during 93 days at their natural moisture content. Description see Fig. 2.

provement in conditions for assimilation, the oxidative component growing only a little. The absence of lag shows the micro-organisms to be in active state. The ratio of oxygen absorbed per glucose consumed by assimilative component is about one. We cannot explain the double maximum curves.

3) After preincubation with diammonium phosphate (variation II) the oxidative component remains practically unaltered except at lowest concentration (Fig. 4, Table 2). A greater number of micro-organisms are in the resting state as indicated by the longer lag. The intermediary state is well separated from the primary oxidation especially by the lowest concentration. As expected the addition of mineral salt resulted in great improvement of conditions for assimilation. The respiration rate during the first steps of assimilation increases according to an exponential curve very similar to that of a growing bacterial culture. The analysis of the respiration curves can be therefore aided by the mathematical expression for this exponential growth. The activity of all kinds of micro-organisms does in fact not increase at the same rate, but we may suppose for the practical comparison of the various curves that the "mean duplication time" T is of some value. This

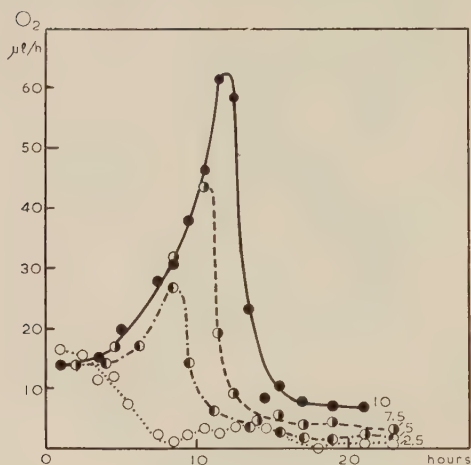


Fig. 4. Respiration rate of soil samples treated with diammonium phosphate and stored in a moist state. Description see Fig. 2.

is the time at which the activity of whole association doubles. The real or apparent extrapolated time of initiation of exponential growth can be designated by τ . There are some organisms – or enzymes – in the sample whose activity still remains constant, and s_k represents their constant respiration rate. The relation of this respiration to the oxidative component needs further elucidation. The equation for exponential course of respiration rate has the following form:

$$\sigma_H = 2^{\frac{1}{T}(H - \tau)} + s_k.$$

σ_H = the respiration rate observed in the time H

T = "mean duplication time"

H = time of experiment

τ = real or extrapolated time of beginning of exponential growth

s_k = constant respiration rate

The respiration rate in the experiment where the sample was preincubated with diammonium phosphate increases according to the equation

$$\sigma_H = 2.95 \cdot 2^{0.366 \cdot H} + 7$$

at an interval of $3 < H < 11.5$ hours. From this expression $T = 2.75$ hours, $\tau = -4.25$ hours, and $s_k = 7$ $\mu\text{l}/\text{hour}$ can be

calculated. These values are valid for both 7.5 and 10 μM of glucose.

4) Drying (variation III) not only destroys the oxidative component (Fig. 5, Table 2) but also alters the assimilative component. The respiration rate increases without an apparent lag. This increase has an exponential course which can be expressed

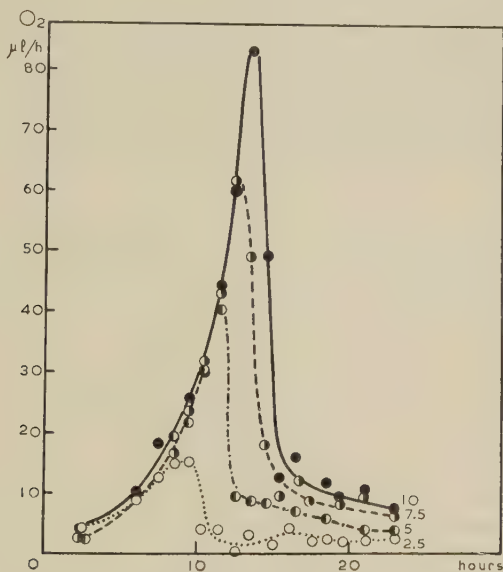


Fig. 5. Respiration rate of the soil samples as in Fig. 4, 24 hours after drying. Description see Fig. 2.

by an equation of a similar form to that of the previous experiment. $T = 2.08$ hours, $\tau = 0$, $s_k = 0$. The shorter duplication time and the absence of constant respiration cause the respiration rate to reach higher maximum than in undried samples. Drying accelerates not only the decay of soil organic matter² but also the assimilation of added substrate. The ratio of oxidation O_2/G diminishes only a little the difference being about 0.1. The experiment was repeated after 6 and 24 days with the same results. It is therefore certain that the dry soil retains its respiratory qualities over at least this period.

5) In spite of all expectations the incubation in anaerobic conditions (variation IV) does not diminish the oxidative component. The results (Fig. 6, Table 2) show that the respiration rate depends, in the first few hours, on the substrate concentration

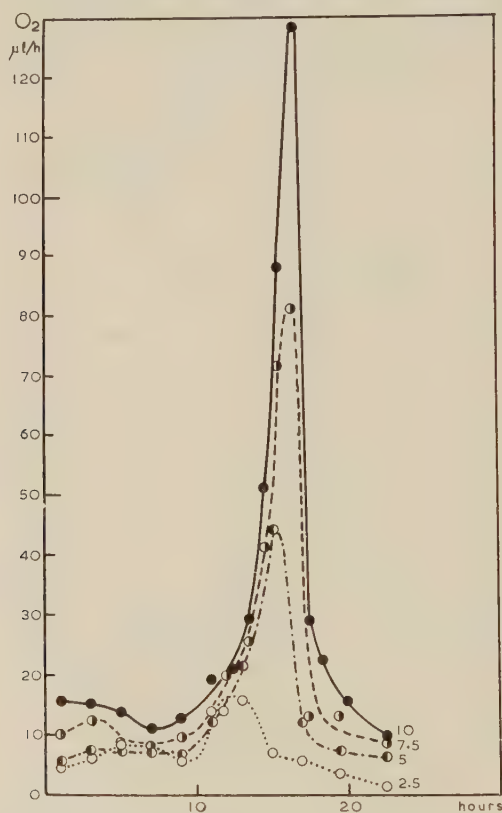


Fig. 6. Respiration rate of the same soil samples as in Fig. 4, after being kept 24 days in anaerobic conditions. Description see Fig. 2.

but decreases slightly before the assimilative stage begins. The conditions for synthesis are very good although the lag phase is prolonged and the respiration curve rises very sharply in the assimilative stage. The parameters which apply for 10 μ M of glucose and $9 < H < 17$ hours: $T = 1.39$ hours, $\tau = +7$ hours, $s_k = 10 \mu$ l/hour. The curves for 5 and 7.5 μ M are similar but shorter with longer lag. Because of short duplication time the

respiration rate reaches an extreme maximum. Also the ratio of oxidation O_2/G is unusually high. It is, therefore, not possible to calculate the ratio of oxidation by assimilative component $(O_2/G)_{ass}$ in the case of lowest concentration because the amount of oxygen consumed by oxidative component is higher than corresponds to the ratio 1.5. For other concentrations the ratio $(O_2/G)_{ass}$ remains constant at about 3.75.

6) Preincubation with rich supply of energy, carbon, and nitrogen sources (variation V) causes a great stimulation of the oxidative component which becomes eight times higher than usual (Fig. 7, Table 2). The assimilative component also has good conditions for development but its expression is limited by the lack

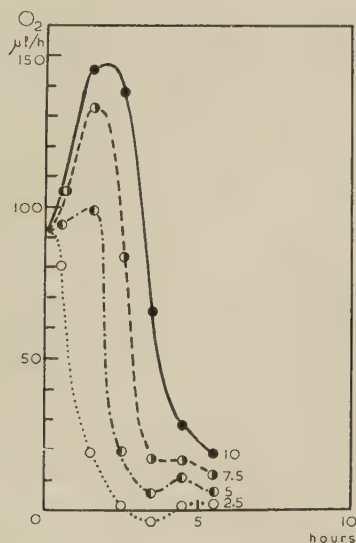


Fig. 7. Respiration rate of soil samples periodically treated with glucose and diammonium carbonate. Description see Fig. 2.

of substrate which is preferably consumed by the oxidative component. The micro-organisms are in an active state as indicated by the absence of lag. The ratio of oxidation O_2/G increases, too, but because the ratio $(O_2/G)_{ox}$ is higher than 1.5 the $(O_2/G)_{ass}$ cannot be calculated. The rate of oxygen consumption by oxidative component does not depend on substrate concentration. The inter-

mediate state is – at the lowest concentrations – well separated from primary oxidation.

From these results it is evident that by influencing the biological component of the soil with various preincubations the character of respiration curves is changed. Provided the micro-organisms remain in an active state the period of lag diminishes or disappears. The oxidative component changes very little. It is destroyed by drying but not by anaerobical conditions of preincubation. The expression of the assimilative component is stimulated by presence of mineral nutrients. It is somewhat stimulated by drying and, very apparently, by anaerobic preincubation. The air-dried sample retains respiratory characteristics for 24 days at least. If conditions favourable for assimilation are present the respiration rate increases exponentially similar to the number of micro-organisms in the culture. The kind of preincubation does influence the ratio O_2/G of primary oxidation, but our experiments do not decide which factor controls this ratio. It is only evident that the relationship of the oxidative to the assimilative component is not decisive in this regard.

SUMMARY

Various kinds of preincubation resulted in great changes in the shape of the respiration curves. The oxidative component is influenced only a little, but it is destroyed by drying. If mineral nutrients are present the increase of the assimilative component results in an exponential increase of respiration rate apparently similar to the growth in number of micro-organisms in a bacterial culture. The "mean duplication time" T is the most important parameter of this process. The ratio of oxygen absorbed to glucose consumed during primary oxidation is also influenced by preincubation – the maximal value being reached after anaerobical preincubation.

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LITERATURE

- 1 Birch, H. F., The effect of soil drying on humus decomposition and nitrogen availability. *Plant and Soil* **10**, 9–31 (1958).
- 2 Corbet, S. A., Studies on tropical soil microbiology: I. The evolution of carbon dioxide from the soil and the bacterial growth curve. *Soil Sci.* **37**, 109–115 (1934).
- 3 Drobník, J., Primary oxidation of organic matter in the soil. I. The form of respiration curves with glucose as the substrate. *Plant and Soil* **12**, 199–211 (1960).

INTERRELATIONSHIPS BETWEEN METHODS OF SPRAY APPLICATION, RETENTION, AND WEATHER CONDITIONS ON THE HERBICIDAL EFFICIENCY OF 2,4-DINITRO-ORTHO-CRESOL

by P. RIEPMA KZN. *

INTRODUCTION

The widespread use of herbicides in agricultural crops is of recent date in comparison with that of fungicides and insecticides. The success of these techniques is dependent on the selective toxicity of the chemicals to different species. However, the divergence between plant species is much less than that between plants and fungi or insects. From field experiments and observations it is known that various plant species react differently to the application of dinitrophenols and synthetic growth regulators (e.g. Blackman, Holly and Roberts ⁴; Roberts and Blackman ²⁵), but up till about 1950 little had been done to define the principles of selective toxicity. In 1952 Blackman ² concluded that specific differences could be dependent on differences in retention, penetration, uptake, transport, local accumulation, and action at cell level. It had also been observed that soil and weather conditions influence herbicidal effectiveness.

Some of the operative factors involved cannot easily be evaluated from data obtained by field experiments, and when the present investigations were being planned in 1953 there was little critical evidence of how far retention coupled with weather conditions would determine the phytotoxicity. Therefore, experiments have been carried out under more controlled conditions, but under conditions which did not depart widely from those in the field, with the object of assessing the influence of spraying techniques

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and meteorological conditions on herbicidal efficiency, in particular of 2,4-dinitro-ortho-cresol.

EXPERIMENTAL METHODS

The experimental techniques employed have been described in detail in former publications (Riepma^{22 23}), so that a short description of the procedures followed will suffice.

The test plants – *Brassica napus*, *Fagopyrum esculentum*, *Pisum sativum* and *Triticum vulgare* – were grown in the open in pots of 25 cm diameter and 10 cm high, and two days before spraying, while the plants were still in the "cotyledon stage", they were thinned respectively to about 50, 30, 25, and 20 plants per pot. The soil consisting of a mixture of fine sand, peat, and compost, was then watered to field capacity. The pots were placed on a travelling endless belt and were exposed to a spray delivered by Duiker-Lyunet nozzles, mounted on a sprayboom in a fixed position 50 cm above the shoots.

For all, save one, experiments which related to measuring phytotoxic effects, 2,4-dinitro-*o*-cresol (DNC) as a suspension of the acid was applied at a constant pressure of 5 kg/cm². The amount of spray liquid applied could be changed by varying the speed of the belt from 15 to 120 m/min and by this procedure the influence of the mean droplet size and the volume applied on the action of DNC could be studied separately.

To calculate the volume of spray applied per hectare it is necessary to know the spray angle of the jet, its output, the speed of the belt and the height of the nozzles above the plants. The spray angle was obtained by photographing the spray cone, illuminated by 2000-Watt lamps, and subsequently enlarging the photographs. The output of water was measured by placing the nozzles in the mouth of a graduated glass cylinder and recording the amount of liquid collected during a fixed time. The speed of the endless belt was determined with a speedometer.

The liquid emerging from a nozzle is shattered into a large number of droplets of varying size and in order to characterize the spray pattern there is a need to know the number of droplets produced in different size classes. The method adopted to do this was as follows: small amounts of spray were allowed to fall into a 1.5-mm layer of Shell diesel oil on a glass plate coated with "drifilm" (a water-repellent silicone). The droplets assumed a spherical form in the oil and were microphotographed; afterwards enlargements were made to facilitate measurements of the diameters. A low surface tension is needed to allow small droplets (diameter of about 10 μ) to penetrate into the oil. Calculations of the volume permitted the mass median diameter to be established (Table 1, Fig. 1).

It is known that the physical properties of liquids influence the pattern of the droplet size (Nukiyama and Tanasawa¹⁸; Lewis *et al.*¹⁷). Therefore, comparative measurements were made of the surface tension (using a Traube stalagmometer) and the viscosity (method of Ostwald) of distilled

water and the DNC suspensions made up from a commercial product containing 50% acid equivalent. No significant differences from water were recorded.

TABLE 1

Some characteristics of the spray produced by different nozzles			
Diameter of nozzle orifice, mm	Spray angle, degrees	Output, l/min	Mass median diameter, μ
1.0	64°54'	0.403	160
1.6	81°2'	0.868	188
2.3	109°6'	1.990	214
2.8	94°8'	3.225	408

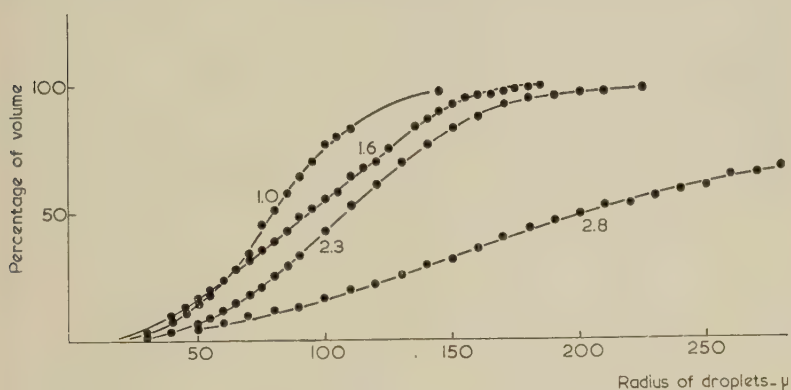


Fig. 1. Distribution of spray-droplet size produced by Duiker-Lyunet nozzles with orifice diameters of 1.0, 1.6, 2.3 and 2.8 mm. Results expressed as a cumulative percentage of the output, the mean mass diameter is given by the 50 percent value.

Retention was determined by spraying the test plants with NaCl solutions (in one case a wetter was added) and immediately after spraying washing the solution off the shoots, cut off at ground level, with distilled water and making up to a standard volume. The resistance was measured by means of a conductivity meter. The amount of liquid retained can be calculated from the linear relationship between the logarithm of the normality of the solution and the corresponding logarithm of the resistance.

This procedure has some disadvantages. There are errors, owing to irregularities in the speed of the endless belt and uneven distribution of the spray pattern in the transverse direction. Moreover, adhering soil particles may be collected in the washings and decrease the resistance. The errors arising from soil contamination were, however, small. In order to minimize other errors twenty plants were used for each separate treatment.

For retention to be put on a quantitative and comparative basis the leaf area must be known. Therefore leaves of *B. napus*, *F. esculentum* and *T. vulgare* were

placed between two glass plates, illuminated from below, and the outline drawn on millimetre graph paper. In the case of *T. vulgare* in addition to the area of the leaves the angle with the vertical was measured with a protractor. As long as the leaves do not overlap, the projected horizontal area may be determined by measuring these quantities. For *P. sativum* the projected horizontal area of entire plants was also measured.

One of the factors controlling retention is the contact angle made between the droplet and the leaf surface and this was determined by the methods described by Fogg¹³ and Hellquist¹⁵. A beam of light, supplied by a Köhler lamp, is directed by means of a plane mirror through the objective and ocular of a horizontal microscope on to a fixed strip of leaf. The image of the droplet on the leaf is projected by a plane mirror placed behind the ocular, on to white paper.

The droplets are shown as dark masses against a light background. Deviations from the spherical form were not observed, so that measurements of base and height sufficed to calculate the contact angle.

Because of the sigmoid relationship between dose and the biological response it is impossible to compare different treatments unless a parameter is introduced which measures and equates the biological effects. The best means of doing this has been statistically examined by a number of workers (e.g. Bliss⁶; Finney¹¹; Blackman²; Sampford²⁶). The greatest amount of statistical information is obtained by converting the dose-response curve into a straight line which is usually achieved by plotting the logarithm of the dose against probit percentage kill.

Ten days after spraying the number of surviving plants was counted. Growth of the axial buds was used as a criterion of survival. Adjustment for natural mortality appeared to be unnecessary so that percentage kill could be calculated without resort to Abbott's formula (see Finney¹¹).

Comparison of different treatments is only valid if the probit lines are parallel. If parallelism does not occur a single value will no longer serve to compare the behaviour of different treatments (Finney¹¹; Sampford²⁶). In consequence, it is necessary to analyse the results to determine whether this parallelism exists which again is tested by standard statistical methods. Parallelism implies that the effect of different treatments on probit percentage kill are independent and additive.

If the regression coefficients of these treatments do not deviate significantly the sum of the logarithms of the independent variates may be used as a single variate (Finney¹¹).

EXPERIMENTAL RESULTS

Factors affecting spray retention

From preliminary experiments on the biological activity of DNC it was observed that next to the contact angle other properties of the spray might influence retention. Therefore, experiments were carried out to assess the influence of the various factors.

The connection between the speed of the belt and the volume applied by different nozzles is summarized in Table 2. If only the speed of the belt is changed, with a few exceptions there is a linear relationship between the volumes applied and retained (Fig. 2 to 6).

TABLE 2

The relation between the speed of the belt and the volume applied per unit area by Duiker-Lyunet nozzles of increasing diameter but at a constant pressure of 5 kg/cm ²				
Speed of belt, m/min	The volume applied (mm ³ /cm ²) with changing diameters of the nozzles			
	Nozzle diameter (mm)			
	1.0	1.6	2.3	2.8
15	4.22	6.77	9.33	20.00
20	3.17	5.08	7.00	15.00
50	1.27	2.03	2.80	6.00
80	0.79	1.27	1.75	3.75
120	0.53	0.89	1.17	2.50

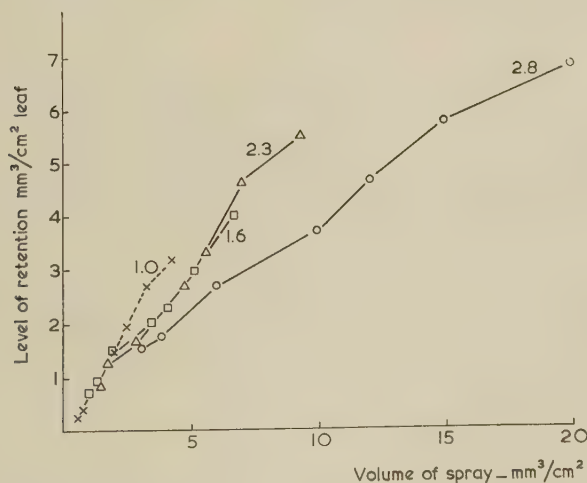


Fig. 2. Expt. 43. *Brassica napus*. The relationship between the level of retention and the volume of spray applied per unit surface, using nozzles with orifice diameters of 1.0, 1.6, 2.3, and 2.8 mm.

From the calculated linear regressions it is found that when they are extrapolated back they pass through the origin. Therefore the slope of the regressions characterizes the relation between retention and output for any particular set of conditions. It is

clear that this linear relation holds both for different mean droplet sizes and for different species, but there is one exception. If the spray liquid is applied with the largest nozzle to *F. esculentum* (Fig. 3) above an output of 15 mm³/cm², there is no further increase in retention.

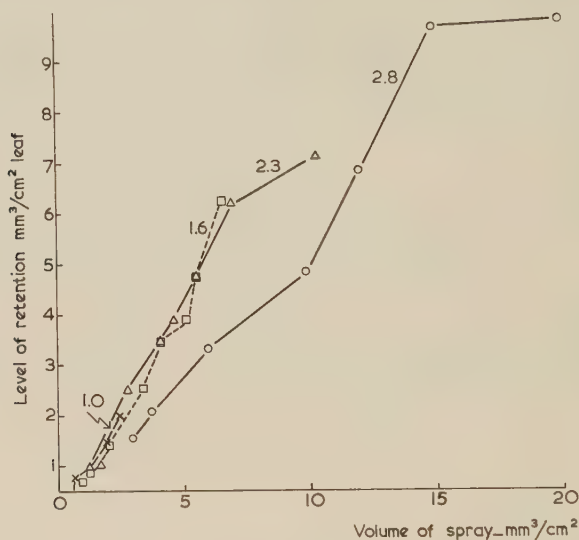


Fig. 3. Expt. 46. *Fagopyrum esculentum*. The relationship between the level of retention and the volume of spray applied per unit surface using nozzles with orifice diameters of 1.0, 1.6, 2.3, and 2.8 mm.

It can therefore be concluded that as long as the spray is applied without change of the mean droplet size or other physical properties, the slope remains constant and may be used to characterize either the influence of droplet size and other physical properties of the spray liquid or differences in leaf surface.

According to Adam¹ and von Eichborn¹⁰ the contact angle alters if the properties of one of the three phases (gas, liquid, and solid) vary. Fogg¹³ and Hellquist¹⁵ have later demonstrated that variations in the characteristics of the leaf surface are reflected in changes in the contact angle. If the leaves are difficult to wet the values of the contact angle will be high and the droplets may bounce or roll off. If the leaves are easily wetted, then, once a continuous film of spray is built up on the surfaces, any further spray will run off the shoot.

The influence of the characteristics of the leaf surface and the angle at which the drops impinge on the leaf is illustrated by the differences in retention of the several species examined. From Table 3 it is evident that over all nozzle sizes the proportion retained by *P. sativum* and *T. vulgare* is less than that remaining on the leaves of *B. napus* and *F. esculentum*. It is also apparent that there is an interaction between species and nozzle output. By and large, as the nozzle diameter is increased and the mean droplet size becomes greater, so the level of retention is reduced in the case of *B. napus* and *P. sativum* and is little changed for *F. esculentum* except when the nozzle exceeds 2.3 mm in diameter. With *T. vulgare*, however, the variation is much more striking.

Some of these specific differences can be ascribed to differences in the wettability of leaves as indicated by the value of the contact angle. Where the wettability is high, as in *B. napus* and *F. esculentum* the degree of retention is greater than for *T. vulgare* and *P. sativum* where the leaves are less readily wetted. Since in Fig. 2, 4, and 5 the relationship between retention and spray output is

TABLE 3

The relation between droplet size, the angle of contact and the ratio of retention to output (with its standard error, in parentheses)						
Species	Exp.	Contact angle (degrees)	Ratio of retention to output			
			Diameter of nozzle orifice, mm			
			1.0	1.6	2.3	2.8
<i>Brassica napus</i>	43	80	0.82 (± 0.03)	0.61 (± 0.01)	0.61 (± 0.015)	0.371 (± 0.015)
<i>Fagopyrum esculentum</i>	46	90	0.765 (± 0.08)	0.835 (± 0.035)	0.81 (± 0.03)	0.56 (± 0.082)
<i>Pisum sativum</i>	76	140	0.34 (± 0.021)	0.27 (± 0.008)	0.16 (± 0.082)	0.20 (± 0.003)
<i>Pisum sativum</i>	81	140	0.29 (± 0.06)	0.205 (± 0.005)	0.245 (± 0.086)	0.215 (± 0.005)
<i>Triticum vulgare</i>	37	140	0.48 (± 0.008)	0.66 (± 0.002)	0.20 (± 0.04)	0.08 (± 0.004)

linear, it is unlikely that differential retention between *B. napus*, *T. vulgare* and *P. sativum* is associated with differences in run-off but for *F. esculentum* the data of Fig. 3 would indicate that with

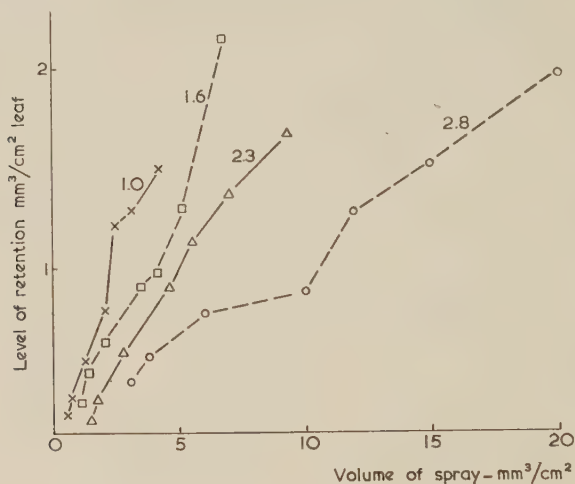


Fig. 4. Expt. 37. *Triticum vulgare*. The relationship between the level of retention and the volume of spray applied per unit surface using nozzles with orifice diameters of 1.0, 1.6, 2.3, and 2.8 mm.

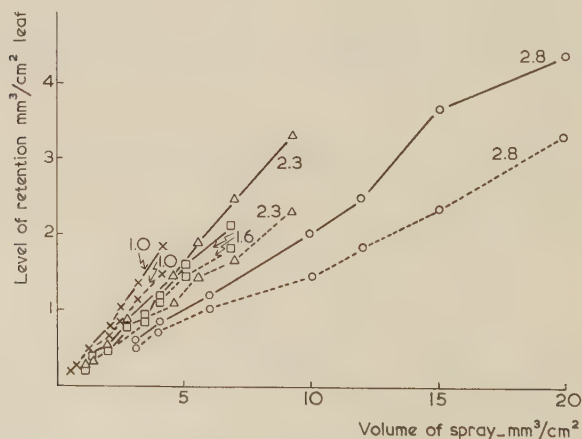


Fig. 5. Expt. 76. *Pisum sativum*. The relationship between the level of retention and the volume of spray applied per unit surface using nozzles with orifice diameters of 1.0, 1.6, 2.3, and 2.8 mm. The relationships for control plants are shown as broken lines and those for plants previously treated with trichloroacetic acid (TCA) as continuous lines.

the two larger nozzles run-off took place following the formation of a continuous film.

There is one further aspect that should be taken into consideration. Brunskill⁷ has shown that the proportion of drops which bounce off the leaf surface of a pea is greatly influenced by the angle of incidence of the impinging drop and in projecting the inclined leaf surface on to a horizontal plane (see p. 243) a complication is introduced in comparing the ratio of retention between different species. The complication is that the proportion of drops which bounce off is dependent on the angle of incidence and though more data are required the available evidence would suggest that the relationships may be curvilinear. Moreover the differences in retention between, say, *B. napus* and *T. vulgare* may only in part be due to the static conditions once the drops have come to rest on the leaf: the dynamic conditions at the moment of impact will also play a part. It seems likely that a far higher proportion of drops bounce off the leaves of *T. vulgare*.

A change in retention can be experimentally induced by altering the waxey nature of the leaf surface (Dewey, Gregory, and Pfeiffer⁸; Pfeiffer, Dewey, and Brunskill²⁰). This change in the character of the cuticle was achieved by applying 6.25 kg/ha trichloroacetic acid (TCA) to the soil before the peas were sown (Exp. 76). By this treatment the contact angle was lowered from 140° to 120° (see Table 4).

The results show that a change in the properties of the leaf surface leads to an increase of the retention coefficient, which is statistically significant if nozzles producing small droplets are used. When large droplets are applied the differences between the retention coefficients at 120° and 140° are not significant.

If the properties of the spray liquid are changed by addition of a surface-active agent (Exp. 81) the ratio between the volumes retained and applied alters too. This change in contact angle from 140° to 30–60° is associated with an increased retention over all nozzle outputs.

Fogg¹³ found that within a single species the contact angle of a leaf alters with age, and with the environmental conditions. He particularly noted that the contact angle of *Sinapis arvensis* changed with the water content of the leaf. In the present investigation the water supply to the roots has also been varied and

TABLE 4

The relation between droplet size, the angle of contact and the ratio of retention to output, (with its standard error, in parentheses) for <i>Pisum sativum</i> . In experiment 76 the contact angle was reduced by pretreatment of the plants with trichloroacetic acid. In experiment 81 a wetting agent was added					
Exp.	Contact angle (degrees)	Ratio of retention to output			
		Diameter of nozzle orifice, mm			
		1.0	1.6	2.3	2.8
76	140	0.34 (± 0.021)	0.27 (± 0.008)	0.16 (± 0.082)	0.20 (± 0.003)
	120	0.42 (± 0.012)	0.30 (± 0.009)	0.34 (± 0.007)	0.22 (± 0.045)
81	140	0.29 (± 0.06)	0.205 (± 0.005)	0.245 (± 0.086)	0.215 (± 0.005)
	30-60	0.34 (± 0.01)	0.293 (± 0.007)	0.29 (± 0.04)	0.23 (± 0.003)

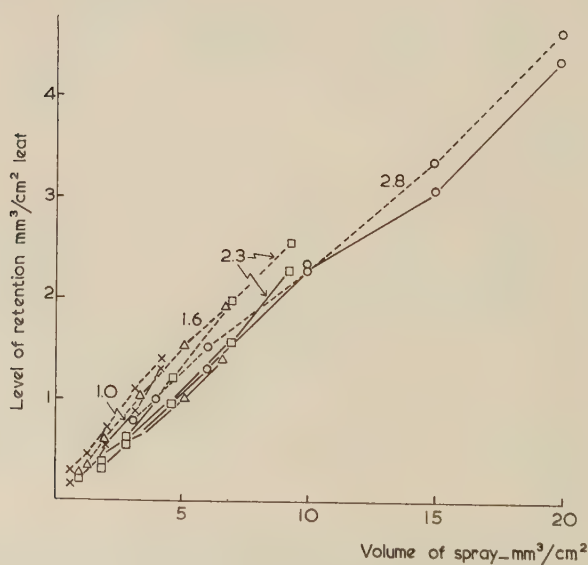


Fig. 6. Expt. 81. *Pisum sativum*. The relationship between the level of retention and the volume of spray applied per unit surface using nozzles with orifice diameters of 1.0, 1.6, 2.3, and 2.8 mm. Treatments where a wetting agent was added to the spray solution are shown as broken lines.

the contact angle measured. *Linum usitatissimum* was initially grown in pots of equal water content, but about a week before the determination of the contact angle the moisture content of the soil was varied by adding varying amounts of water to the pots. No uniformity of the distribution of the water in the soil was attempted, the aim being to simulate the effect of rainfall.

TABLE 5

The influence of the moisture content of two types of soil on the contact angle of <i>Linum usitatissimum</i>			
Soil type	Moisture content (v/w %)	Contact angle (degrees)	Standard error of contact angle
Clay	18.7	122°50'	2°18'
	24.3	118°20'	2°30'
	35.5	105°14'	2°42'
Peat	29.2	122°13'	1°42'
	40.4	122°12'	2°—
	51.7	113° 5'	1°38'
	62.9	98°55'	2°—

The data of Table 5 confirm that the water supply to the roots of *L. usitatissimum* influences the contact angle of the leaves and thereby the ease of wetting.

Thus, when the leaves are fully turgid they will be more easily wetted. Other experiments with *Stellaria media* and *Phaseolus vulgaris* confirmed these results.

The influence of the level of retention on the biological response

In addition to the experiments concerned with the factors determining retention, the influence of varying retention on the phytotoxic effects of 2,4-dinitro-*o*-cresol (DNC) was investigated. Clearly, for a given concentration of the herbicidal spray the dose received by the plants depends on the amount of spray retained, which is dependent on the volume applied and the retention coefficient, which in its turn is linked with the droplet size (Table 3).

Normally percentage kill increases in a sigmoid fashion with the increase of the concentration provided that the other factors including the spraying technique are kept constant. Varying one of these factors could well influence the concentration required to cause a standard biological response and therefore experiments have been carried out to investigate these interrelationships.

If the volume of spray applied is altered over a range such that the level of retention is still linearly related to output then it could be advanced that the equi-effective concentration required to bring about a standard response would be inversely and linearly proportional to the volume of spray applied. Thus, if these relationships hold and there are no interactions the probit lines for each level of output should be parallel. Parallelism can be established by employing the methods put forward by Finney¹¹.

This procedure has been applied to the results of an experiment with *F. esculentum* in which the nozzle orifice was 1.6 mm and the outputs at the three band speeds were 4.06, 2.03, and 1.35 mm³/cm². Inspection of Fig. 7 suggests that the probit lines are parallel and this is confirmed by statistical analysis. It is also apparent that as the output is increased so the equi-effective concentration is reduced.

The results of a further experiment employing another range of output (5.60, 2.80 and 1.87 mm³/cm²) are given in Fig. 8 and statistical analysis showed that the probit lines for the three outputs are again parallel and that there is an inverse relationship between output and the equi-effective concentration. To demonstrate that this relationship is linear the probits of percentage kill

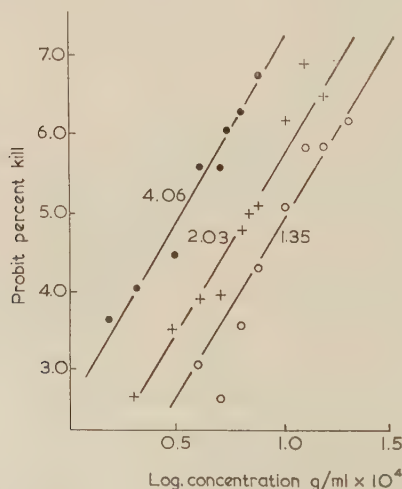


Fig. 7. Expt. 75. *Fagopyrum esculentum*. Regressions of probit kill against the logarithm of the concentration of dinitro-*o*-cresol for different spray outputs - 1.35, 2.03, and 4.06 mm³/cm².

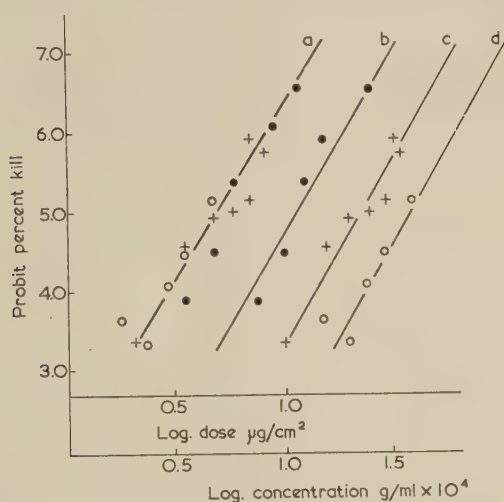


Fig. 8. Expt. 29. *Fagopyrum esculentum*. Regressions of probit kill against the logarithm of (a) dose and (b), (c), and (d) the concentrations of dinitro-*o*-cresol required for different spray outputs of 5.60, 2.80, and 1.87 mm³/cm².

TABLE 6

The relationship between nozzle output and the dose required to bring about a 50% kill of <i>B. napus</i> by dinitro- <i>o</i> -cresol, when the coefficient of retention is 0.823 and the nozzle orifice is 1.0 mm			
Speed of belt (m/min)	Volume of spray applied (mm ³ /cm ²)	Concentration of DNC for 50% kill (g/100 ml)	Dose for 50% kill (μg/cm ² leaf)
25	2.53	0.296	5.91
50	1.27	0.689	7.10
100	0.64	0.980	5.02

have also been plotted against dose where dose has been derived by multiplying together the concentration of DNC, the spray output and the relevant coefficient of retention (see Table 3). It is clear that all the data can be satisfied by a single regression.

Similar experiments were undertaken with *B. napus* and the data of Table 6 confirm that for this species also the equi-effective concentration is inversely related to the amount of spray retained. Further confirmation comes from other experiments (Fig. 9) where it is seen that for three different nozzles there is a linear relationship between the equi-effective concentration and the reciprocal of the spray output.

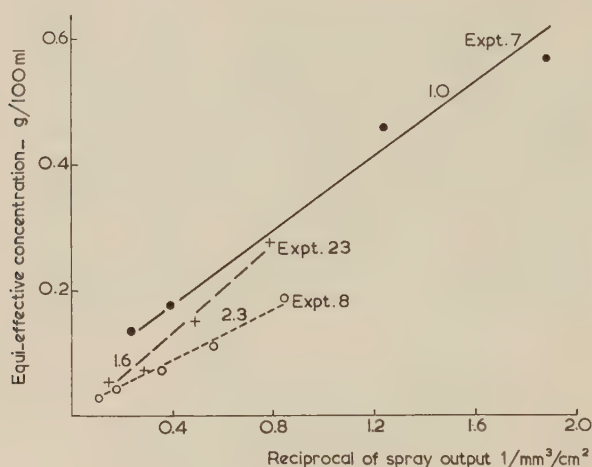


Fig. 9. *Brassica napus*. The relationship between the equi-effective concentration of dinitro-*o*-cresol and the reciprocal of spray output using nozzles of orifice diameters 1.0, 1.6, and 2.3 mm.

The data of Table 6 and Fig. 7, 8, and 9 would suggest that the linkage between the degree of kill and the amount of herbicide received by the plant was independent of the mean droplet size since though different jets were used in the several experiments the general trend was the same. Since, within these experiments there were no direct comparisons of different nozzles, further experiments with *B. napus* were undertaken.

Examination of Table 7 confirms that a change in the mass median diameter of the spray droplets resulting from an alteration in jet size is not an important factor in determining the equi-effective dose of dinitro-*o*-cresol. Between treatments there is some variation in the dose required but it must be borne in mind that the retention factors employed to calculate the dose in this and the previous experiments were not directly determined at the same time, but were derived from other experiments. Thus, some errors may be introduced in extrapolating the coefficient of retention from one experiment to another.

Some measure of these errors can be obtained from other experiments where in individual tests the equi-effective concentrations of DNC were determined for two different nozzles at the three outputs obtained by employing throughout band speeds of 25, 50, and 100 m/min. Thus, by averaging the data for the three

TABLE 7

The relationship between nozzle output and the dose required to bring about a 50% kill of <i>B. napus</i> by dinitro- <i>o</i> -cresol when nozzles of different size are employed				
Speed of belt	Nozzle orifice diam.	Volume of spray applied	Concentration of DNC for 50% kill	Dose for 50% kill
(m/min)	(mm)	(mm ³ /cm ²)	(g/100 ml)	(μg/cm ² leaf)
25	2.3	5.60	0.056	1.92
50	2.3	2.80	0.135	2.29
100	2.3	1.40	0.309	2.62
25	2.8	12.00	0.065	2.90
50	2.8	6.00	0.092	2.04
100	2.8	3.00	0.212	2.35

outputs for each nozzle a mean figure of the quantity of DNC required per unit surface to give a 50% kill of *B. napus* is obtained. If the retention coefficients for the two individual nozzles remain constant between experiments then the ratio of the quantity of DNC required for any two nozzles should also remain constant. Inspection of Table 8 shows that for the nozzles with orifice diameters of 2.3 and 2.8 mm there is a good agreement between experiments and the average ratio over all experiments – 1.53 – does not differ markedly from the ratio of 1.64 which would be expected from the results cited in Table 3. For the two smaller jets the data are not so consistent, nevertheless the average ratio is 1.17 and that expected 1.34.

It has already been demonstrated that the retention coefficient

TABLE 8

The influence of nozzle size on the quantity of dinitro- <i>o</i> -cresol (μg/cm ²) which had to be applied per unit surface to cause a 50% kill of <i>B. napus</i>			
Expt. no	Nozzle orifice diam.		Ratio
	2.3 mm	2.8 mm	
33	1.68	2.18	1.30
49	4.17	6.57	1.58
52	7.03	11.35	1.61
62	3.27	5.32	1.63
78	3.04	4.69	1.53
	1.0 mm	1.6 mm	
50	5.47	6.53	1.20
77	5.32	5.16	0.98
53	7.71	10.30	1.34

is linked with the contact angle and a further experiment was undertaken to investigate the reactions of pea plants, previously treated with trichloroacetic acid. In this instance the plants were sprayed with 2,4-dinitro-6-secondary-butylphenol (DNBP), using the same nozzle throughout (diameter 2.3 mm).

Prior to sowing the peas the soil was treated with 0 and 6.75 kg/ha trichloroacetic acid and at the time of spraying the values of the contact angles were 120° and 140° for the treated and control plants, while the corresponding retention coefficients were 0.32 and 0.24. In Fig. 10 the probit values of the percentage reduction in

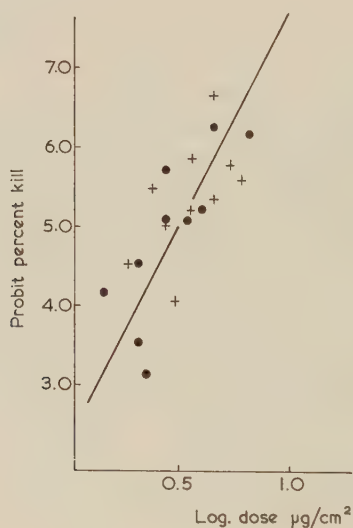


Fig. 10. Expt. 79. *Pisum sativum*. Regressions of probit kill against the dose of 2,4-dinitro-6-secondary-butyl-phenol for control plants (+) and plants previously treated with trichloroacetic acid (•).

the growth rate have been plotted against dose calculated from the concentration of phenol in the spray solution, the nozzle output and the coefficient of retention. Inspection shows that there is an equal scatter of points on each side of a single regression for control plants and those pretreated with trichloroacetic acid. Thus the data would suggest, but not conclusively, that the pretreatment has operated largely by altering the coefficient of retention and any induced change in the cuticle has not affected the degree of penetration of DNBP.

*The influence of environmental factors on the phytotoxicity of 2,4-dinitro-*o*-cresol*

Under commercial conditions of spraying the weather is an uncontrollable variable and allowances for weather conditions can rarely be made with certainty. During the course of the present experiments some information has been gained on the extent to which the equi-effective concentration varies with the weather conditions at the time of spraying. Such variation in the equi-effective concentrations could also be due to changes in retention dependent on the stage of development or other morphological variables.

To minimize errors due to plant differences the aim was to select in all the experiments test plants of a uniform stage of development. To gain further information on the uniformity of the populations, the mean leaf area as well as the contact angle were determined. It was assumed that if the contact angle ranged between 75° and 85° in the experimental batches of *B. napus*, then the variations in the level of retention would be small. Thus the amount retained for a given spray output could be calculated from the appropriate ratio of retention to output determined in earlier experiments (see Table 3).

The initial selection for uniformity between experiments was that the leaf area per plant should be 3.5 cm^2 , and this stage was judged by eye. Subsequently the mean leaf area of the plants used in any one experiment was measured and the amount of spray retained expressed as mm^3/cm^2 . To obtain the equi-effective dose of DNC, the amount of spray retained was multiplied by the concentration causing a 50% mortality at the end of ten days.

On the basis of existing knowledge the main factors likely to influence the phytotoxic action at the time of spraying would be the solar radiation, the mean temperature and the difference between mean and dew-point temperatures (calculated from the saturation deficit of the air) since all these factors will affect the rate of drying of the spray solution.

Subsequently, once the compound had penetrated into the plant, it seemed most likely that its action would be dependent on solar radiation and temperature. Data for solar radiation, temperature and saturation deficit were obtained from the meteorological sub-station at Wageningen some 2 to 4 kilometres away.

The results of fifteen experiments carried out at different times of the year with *B. napus* are set out in Table 9. The dose cited in the table is the mean of 4 to 10 figures obtained for each of the spray treatments in the separate experiments. It was observed that between the experiments the time taken for the spray to dry out on the leaf surfaces ranged from 10 to 30 minutes. It seemed therefore of interest to examine first the relationship between dose and the meteorological conditions at the time of spraying. The graphical record of the changes in solar radiation did not permit of an accurate estimate for less than a period of an hour and estimates over hourly intervals starting at the time of spraying were made. In Table 9 the meteorological data include the solar radiation, the mean temperature, and the difference between the mean and the dewpoint temperatures for the hour of spraying operation, together with the mean temperature over the 10 days before the counts of mortality were made.

It would be expected that the level of solar radiation would be

TABLE 9

The interrelationships between the dose of dinitro- <i>o</i> -cresol required to cause a 50% mortality of <i>B. napus</i> , the solar radiation, the mean temperature, the difference between mean temperature and dewpoint temperature during the hour after spraying took place and the mean temperature for the 10 days subsequent to spraying							
Expt. no.	Nozzle orifice mm	Range of spray output mm ³ /cm ²	Dose μg/cm ²	Radiation cal/cm ² min	Temperature °C	Difference temperature and dew-point temperature °C	Mean temperature 10 days °C
9	2.3	1.8- 9.3	1.25	0.041	13.5	3.9	12.5
33	1.0, 1.6, 2.3, 2.8	1.3-12.0	1.31	0.365	13.0	4.4	15.5
8	2.3	1.9- 9.3	1.39	0.148	12.0	3.9	11.5
78	2.3, 2.8	1.4-12.0	1.81	0.279	11.5	3.1	9.5
62	2.3, 2.8	1.4-12.0	1.95	0.269	20.0	8.8	19.0
23	1.6	1.3- 6.8	2.01	0.288	10.5	2.8	6.5
49	2.3, 2.8	1.4-12.0	2.29	0.211	11.0	3.6	8.5
77	1.0, 1.6	0.6- 4.1	3.49	0.691	14.5	4.4	13.0
7	1.0	0.5- 4.2	3.70	0.384	13.0	4.0	14.0
52	2.3, 2.8	1.4-12.0	4.08	1.190	19.0	10.8	14.5
5	1.6	2.0- 7.3	4.50	0.960	19.0	8.5	14.5
50	1.0, 1.6	0.6- 4.1	4.76	1.094	13.5	5.7	12.0
57	1.0, 1.6	0.6- 2.5	5.21	0.902	19.0	8.7	15.0
53	1.0, 1.6	0.6-4.1	6.52	0.998	15.0	5.7	12.0
54	2.3, 2.8	3.0-12.0	8.21	1.209	21.0	10.3	16.0

highly correlated with mean temperature and the difference between mean temperature and dewpoint temperature would, in turn, be correlated with temperature. That this is so is evident from Table 10. Nevertheless, it is equally clear that the dose required to bring about a 50% kill is positively and significantly correlated with solar radiation and rather less so with temperature and the difference between mean and dewpoint temperature.

The linkage between dose and radiation is further brought out in Fig. 11 where it would seem that the data are satisfied by fitting a linear regression.

TABLE 10

Correlation coefficients between the dose of dinitro- <i>o</i> -cresol required to cause a 50% mortality of <i>B. napus</i> , the solar radiation, the mean temperature and the difference between mean temperature and dewpoint temperature during the hour of spraying and the mean temperature for the 10 days before the mortality counts were made				
Variables	Radiation	Mean temp. (hour)	Diff. from dewpt. temp.	Mean temperature (10 days)
Dose	+0.87 *	+0.61 *	+0.62 *	+0.29
Radiation		+0.64 *	+0.72 *	+0.50
Mean temperature (hour)			+0.96 *	+0.82 *

* Denotes correlation significant at $P = 0.05$.

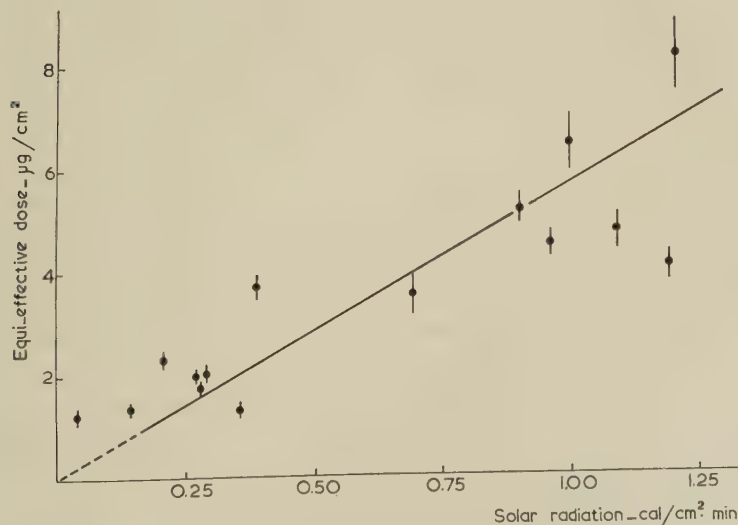


Fig. 11. *Brassica napus*. The relationship between the dose of dinitro-*o*-cresol required to bring about a 50% kill and the solar radiation received during the hour following the spray application.

From Table 10 it is also to be noted that though the mean temperature over the ten days intervening before counts were made is significantly correlated with the mean temperature during the hour of spraying it has little influence on the level of dose. This lack of significant effect would suggest that it is the meteorological conditions at or shortly after spraying which determined the ultimate phytotoxic effect. To confirm this assumption, further correlations were calculated between dose and solar radiation and mean temperature for the first seven hours after spraying and these are given in Table 11. Once more it is apparent that temperature is less important than radiation.

TABLE 11

The correlation coefficients between the dose of dinitro- <i>o</i> -cresol required for a 50% mortality of <i>B. napus</i> and (a) the mean radiation received and (b) the mean temperature during the first seven hours after spraying						
Variables	Period after spraying (hours)					
	0-2	0-3	0-4	0-5	0-6	0-7
Radiation	0.86 *	0.82 *	0.78 *	0.74 *	0.71 *	0.67 *
Temperature	0.45	0.35	0.27	0.23	0.21	0.18

* Significant at $P = 0.05$.

As a further confirmation that the effect of temperature after a few hours is small, correlation coefficients for dose and the mean temperature over 2, 4, 6, 8, and 10 days were also calculated and all failed to attain a level of significance.

DISCUSSION

As stated in the introduction the aim of this investigation has been to assess to what extent the type of spray application and the weather conditions influence the dose needed to cause a standard biological response. From the present experiments it is evident that the ratio between the level of retention and the spray output remains relatively constant as long as the output is altered only by a change in band speed. There was however one exception: namely with *F. esculentum* the ratio fell when the output exceeded $15 \text{ mm}^3/\text{cm}^2$, see Fig. 3. In this connection it is of interest to note that in the investigations of Blackman, Bruce and Holly³ as long as the surface tension was high the amount

retained by *P. sativum* and *Hordeum vulgare* was linear related to spray output up to a level of $14.3 \text{ mm}^3/\text{cm}^2$. On the other hand for *Helianthus annuus* the linearity ceased at about $10 \text{ mm}^3/\text{cm}^2$, and for *Brassica alba* the proportionality only held up to $5 \text{ mm}^3/\text{cm}^2$. Similarly Hellquist¹⁵ recorded that for *Chenopodium album* and *Sinapis arvensis* the proportion of the spray retained did not greatly alter when the spray output ranged from 50 to 350 litres/ha (equivalent to 0.5 to $3.5 \text{ mm}^3/\text{cm}^2$). When the output was larger the ratio decreased.

It has also been demonstrated that as long as the output does not exceed circa $12 \text{ mm}^3/\text{cm}^2$, then the slope of the regression linking retention with output is characterised by the mean droplet size and the species.

In general the ratio between the volumes retained and applied decreases with an increase in the mass median diameter of the spray drops. These results are in agreement with those of Bruns-kill⁷, who observed that, when pea leaves were fixed in a horizontal position and drops of an uniform but variable size were allowed to fall on them, the retention increased with a decrease in droplet size. Thus the decrease in the retention coefficient resulting from a change to a larger nozzle (Fig. 2 to 4) must be ascribed to the greater mean diameter of the spray droplets.

This investigation has confirmed that there are large differences in retention between species and it would seem that *B. napus* and *F. esculentum* belong to the class where most of the drops remain on the leaf surface even if the surface tension is high. For these species lowering the surface tension in general lowers the level of retention. *Pisum sativum* is in a different category since as the surface tension is reduced retention is increased because the droplets are less liable to bounce off the leaves. Thus, the present results are in agreement with those of Blackman, Bruce, and Holly³.

It has been found that the retention coefficient increases as the contact angle decreases, but though the contact angles for peas and wheat are the same, the retention coefficient for wheat is smaller when the mean droplet size is large. This specific difference is ascribed to the variation in the angle at which the drops impinge on the leaves. When the drops are small the difference in slope between a pea and wheat leaf affects retention less than when the drops are large.

Another factor which lowering of the surface tension brings into play is the decrease in the mass median diameter (Nukiyama and Tanasawa¹⁸; Lewis *et al.*¹⁷). Thus surface tension can in part alter the level of retention by altering drop size.

Besides the specific differences in the nature of the leaf surface and the physical characteristics of the spray droplets which will determine the contact angle and thereby the level of retention, there is a third group of physiological factors which may be involved. Since the present experiments on *Linum usitatissimum* have confirmed Fogg's findings¹³ for *S. arvensis* that the contact angle is linked to the water supply to the leaves it seems reasonable to suppose that in the field retention will be to some extent dependent on the water status of the leaves. Thus under conditions of an internal water deficit the retention coefficient will be lowered. These conditions are likely to arise if a high level of solar radiation and a dry atmosphere induce a high rate of transpiration at a time when the soil is drying out.

Although it has proved possible to define and itemize the factors which determine the retention coefficients of some species no precise assessment of the extent to which specific differences in retention operate under field conditions can be attempted. As Blackman, Bruce, and Holly³ have emphasised the amount retained changes with the stage of development and the nature of the interaction is dependent on the species and the physical characteristics of the spray application. However, the evidence now accumulating from this and other studies of retention should allow some prediction of what type of spray application is likely to bring about the biggest differences in retention between wanted and unwanted species.

Turning to the interrelationships between retention and dose the present experiments have consistently demonstrated that the dose of DNC needed for a standard degree of mortality remains constant irrespective of the variations in droplet size or the volume of spray retained. Thus in these experiments the equi-effective concentration of DNC is inversely and directly correlated with the absolute amount of spray retained. On the other hand, Blackman, Bruce, and Holly³ record that this inverse relationship was linear only when a freely translocated herbicide, such as 2,4-D was employed and that for the ammonium salt of DNBP, a

“contact” herbicide, the relationship was logarithmic. There was however evidence that the linkage between the equi-effective concentration of DNBP and the amount retained varied with the species. It should also be emphasised that the plants were well past the cotyledon stage at the time of spraying. Thus, some inconsistency between the two sets of findings is not surprising.

It has already been stressed that in interpreting the effects of meteorological conditions on the phytotoxic activity of DNC a distinction must be made between the effects on the processes governing penetration and changes induced in the physiological activity after entry.

All the evidence suggests that under the experimental conditions of this investigation it is the conditions at the time of spraying – particularly radiation – which are most important in determining the final toxic action on seedlings of *B. napus*. It is well established that the inhibition of enzyme activity by nitrophenols is temperature sensitive and there is direct evidence (Fogg¹⁴; Meggitt, Aldrich, and Shaw¹⁹) that rising temperature leads to enhanced injury of shoots by DNC. On this basis one should expect a negative correlation between the equi-effective dose and temperature. The fact that the correlation is positive in the present experiments leads to the conclusion that the rate of drying out is more important because a slow rate of drying will allow more DNC to penetrate. These results in some respects run counter to the experiences of spray contractors in England who often reduce the concentrations of the nitrophenol if the temperature exceeds circa 18°C. This practice contrasts with the recommendations given in the Netherlands (Riepma^{23 24}). Moreover there is evidence that the concentration of DNC should be lowered if it is anticipated that frost or even night frost may occur within a few days of spraying (Pfeiffer²¹; Eggink and Riepma⁹). Some of these disparities may be associated with the exact ranges of temperature involved. Meggitt, Aldrich, and Shaw¹⁹ found no significant influence of temperature until circa 21°C was reached. In most of the present experiments the temperature only rarely rose above 21°C.

Another complexity which needs to be considered is the possible variation in the susceptibility of the test plant due to the meteorological conditions before spraying. Clearly if the solar radiation

and temperature at the time of spraying is high it is likely, that the seedlings will have been raised under conditions where radiation and temperature have also been high and conversely low levels of radiation and temperature at the time of spraying are likely to be associated with plants which will have been subjected to similar conditions during their previous growth. As a result there may be considerable morphological differences between the batches of plants used in the different experiments even though the contact angle may be of the same order. Evidence for this may be found in the data of Hull¹⁶, who studied the effect of growth conditions on the wax formation by mesquite (*Prosopis juliflora* L.). For example temperature and radiation may well alter not only the structure, but also the nature of the surfaces and this in turn will alter the rate of penetration.

Apart from the effects of the environment before spraying on the morphological characters there can also be alterations in the physiological status. According to Blackman and Robertson-Cunninghame⁵, young plants of *Helianthus annuus* exposed to full daylight prior to spraying with 2,4-dichloro-5-iodophenoxy-acetic acid retained a little less than plants which had received 0.12 daylight.

On the other hand, penetration into leaves was not greatly affected by the level of light the plants were given before treatment. In this instance the duration of pretreatment was only for three days. If the sunflowers were grown under different light intensities from emergence to the time they were sprayed then plants receiving 0.12 daylight were 1.6 times more sensitive to 2,4-D than those grown in full daylight.

SUMMARY

Employing four test plants (*Brassica napus*, *Fagopyrum esculentum*, *Pisum sativum* and *Triticum vulgare*) the effects of varying the spray output (0.55 to 20.0 mm³/cm²) and the nozzle orifice (mean mass diameter of spray droplets 160–408 μ) on retention by the shoots of young seedlings were first investigated. As long as the nozzle output did not exceed 15 mm³/cm², then for each species and for each nozzle the level of retention was proportional to the volume of spray reaching the leaf surface. For a given output the proportion retained was inversely related to the mean droplet size. The magnitude of this effect was greatest for *T. vulgare* and least for *P. sativum*.

Lowering the surface tension of the spray solution or pretreating plants of *P. sativum* with trichloroacetic acid diminishes the contact angle made between the drops and the leaf surface and this reduction in the angle is associated with a higher retention ratio.

The relation between the amount of spray retained and the concentration of 2,4-dinitro-*o*-cresol (DNC) required to kill half the plants of *F. esculentum*, *P. sativum* and *B. napus* was studied. For each species, irrespective of spray output or mean droplet size the equi-effective dose of DNC remained constant *i.e.* the equi-effective concentration was constantly and inversely proportional to the level of retention per unit surface.

Statistical analysis of a series of experiments undertaken at different seasons of the year on *B. napus* demonstrated that the magnitude of the equi-effective dose of DNC was primarily dependent on the total solar radiation received during the first few hours after the spray application.

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REFERENCES

- 1 Adam, N. K., The Physics and Chemistry of Surfaces. Oxford Univ. Press (1942).
- 2 Blackman, G. E., Studies in the principles of phytotoxicity. I. The assessment of relative toxicity. J. Exptl. Botany **3**, 1-27 (1952).
- 3 Blackman, G. E., Bruce, R. S. and Holly, K., Studies in the principles of phytotoxicity. V. Interrelationships between specific differences in spray retention and selective toxicity. J. Exptl. Botany **9**, 175-205 (1958).
- 4 Blackman, G. E., Holly, K. and Roberts, H. A., The comparative toxicity of phytocidal substances. Symposia Soc. Exptl. Biol. **3**, 283-317 (1949).
- 5 Blackman, G. E. and Robertson-Cunninghame, R. C., Interrelationships between light intensity and the physiological effects of 2,4-dichloro-phenoxyacetic acid on the growth of *Helianthus annuus*. J. Exptl. Botany **6**, 177-211 (1955).
- 6 Bliss, C. I., The method of probits. Science **79**, 38-39 (1934).
- 7 Brunskill, R. T., Physical factors affecting the retention of spray droplets on leaf surfaces. Proc. Brit. Weed Control Conf. 3rd Conf., 593-603 (1956).
- 8 Dewey, O. R., Gregory, P. and Pfeiffer, R. K., Factors affecting the susceptibility of peas to dinitroherbicides. Proc. Brit. Weed Control Conf. 3rd Conf., 313-326 (1956).
- 9 Eggink, H. J. and Riepma Kzn, P., De voor onkruidbestrijdingsmiddelen gevoelige stadia van graangewassen. Verslag Centraal Inst. Landbouwk. Onderzoek **1950**, 121-128 (1951).
- 10 Eichborn, J. L. von, Zur Frage des gegenseitigen Haftens räumlich nicht mischbarer Stoffe. Probleme der Randwinkel insbesondere von Flüssigkeiten an Festoberflächen. Kolloid Z. **107**, 107-113 (1944).

- 11 Finney, D. J., Probit Analysis. Cambridge Univ. Press (1952).
- 12 Fisher, R. A. and Yates, E., Statistical Tables for Biological, Agricultural and Medical Research. Oliver and Boyd, Edinburgh (1948).
- 13 Fogg, G. E., Quantitative studies on the wetting of leaves by water. Proc. Roy. Soc. London B **134**, 503-522 (1947).
- 14 Fogg, G. E., The penetration of 3,5 dinitro-*o*-cresol into leaves. Ann. Appl. Biol. **35**, 315-330 (1948).
- 15 Hellquist, H., The effect of volume application rate on the retention and activity of herbicides. Kgl. Lantbruks-Högskol. Ann. **22**, 41-92 (1955).
- 16 Hull, H. M., The effect of day and night temperature on growth, foliar wax content and cuticle development of velvet mesquite. Weeds **6**, 133-142 (1958).
- 17 Lewis, H. C., Edwards, D. G., Goglia, M. J., Rice, R. I. and Smith, L. W., Atomization of liquids in high velocity gas streams. Ind. Eng. Chem. **40**, 67-74 (1948).
- 18 Nukiyama, S. and Tanasawa, Y., An experiment on the atomization of liquid by means of an airstream. Trans. Soc. Mech. Engrs. Japan. **4**, 86-94 and 138-143 (1938) and **5**, 63-67 (1939).
- 19 Meggitt, W. F., Aldrich, R. J. and Shaw, W. C., Factors affecting the herbicidal action of aqueous sprays of salts of 4,6-dinitro-ortho-secondary butylphenol. Weeds **4**, 131-138 (1956).
- 20 Pfeiffer, R. K., Dewey, O. R. and Brunskill, R. T., Further investigations on the effect of pre-emergence treatment with trichloroacetic and dichloropropionic acids on the subsequent reactions of plants to other herbicidal sprays. Mimeo. Rept. Fisons Pest Contr. Ltd. Cited according to Weed Abstracts **1957**, no. 606 (1957).
- 21 Pfeiffer, R. K., Gregory, P. and Holmes, H., The use of dinitro weedkillers on winter cereals. Proc. Brit Weed Control Conf. 2nd Conf., 433-445 (1954).
- 22 Riepma Kzn, P., Spuittechniek en weersomstandigheden Landbouwk. Tijdschr. **66**, 99-107 (1954).
- 23 Riepma Kzn, P., Grensvlakverschijnselen en selectiviteit. I. Methoden van onderzoek. Verslag Centraal Inst. Landbouwk. Onderzoek 1954, 210-214 (1955).
- 24 Riepma Kzn, P., Chemische onkruidbestrijding in granen. (spuittechniek en weersomstandigheden). Landbouwvoorlichting **12**, 128-133 (1955).
- 25 Roberts, H. A. and Blackman, G. E., Studies in selective weed control. III. The control of annual weeds in leguminous crops with 2,4-dinitro-6-secondary butylphenol. J. Agr. Sci. **40**, 263-274 (1950).
- 26 Sampford, M. R., Studies in the principles of phytotoxicity. II. Experimental designs and techniques of statistical analysis for the assessment of toxicity. J. Exptl. Botany **3**, 28-46 (1952).

THE ACTION OF 4,6-DINITRO-*o*-CRESOL (DNOC) IN SOIL

I. THE EMERGENCE FROM CRESS SEED IN DNOC-TREATED SOIL MIXTURES

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The herbicide 4,6-dinitro-*o*-cresol (DNOC) inhibits the emergence of garden cress (*Lepidium sativum* L.) seedlings from soil ¹⁵. The more time passes between the addition of DNOC to the soil and the sowing, the more the emergence percentage increases. After some weeks it surpasses that of control samples and mounts to $1\frac{1}{2}$ times the control value. It was concluded that minute amounts of DNOC or some breakdown product of DNOC stimulates emergence ¹⁵.

It is the intention of the present paper to investigate into the cause of this enhancing effect of DNOC treatment of soil on the emergence percentage of cress seedlings.

METHODS

A. Emergence experiments

A soil mixture was prepared by mixing sieved parts of vegetable mould, sand and peat dust in the ratio of 20 : 20 : 1 respectively, on a dry weight basis. For a number of experiments the mixture was sterilized by autoclaving for 30 minutes at 120°C.

Flower pots of 200 ml capacity were internally made impervious to water with Parawet (Chem. Ind. "Den Haag") and the hole in the bottom was covered with a disc of cellophane sheet.

Portions of 200 g of the soil mixture were each thoroughly mixed with 30 ml of a watery DNOC solution (pure sodium salt) or, in case of the controls, with 30 ml of distilled water. The portions were next transferred into the flower pots, in which the soil was lightly packed. The pots were kept at 25°C in the dark in an atmosphere of air, saturated with water, throughout the experiments. Before sowing, a bit of the soil was removed,

100 selected seeds were uniformly distributed and next covered with the bit of soil. The emergence was determined after 2, 3, and 5 days. At the end of the experiments, the lengths of root and shoot of the seedlings could be measured.

B. Soil extraction

Soil from 3 pots was shaken with 500 ml of ethanol, 96%, or water, during 1 h at 100 strikes per min. After the settling down of the sediment, the supernatant liquid – about 400 ml – was decanted and cleared by centrifugation at $35,000 \times g$ during 15 min at 10°C, followed by filtration of the supernatant through a Buchner funnel to remove floating peat particles. The ethanolic extract was evaporated to dryness and the watery one reduced to 100 ml at room temperature *in vacuo*. The residue of the ethanol extract was taken up in 100 ml of water.

Of these 100-ml watery solutions, 90 ml were used in the emergence test, *viz* 30 ml per pot, whilst the remaining 10 ml were diluted to 25 ml and served for identification purposes.

C. Paper chromatography

Descending paper chromatography was performed on sheets of Whatman No. 1 filter paper, 40 cm $\times$ 44 cm. The lower margin of the sheet was notched to allow the solvent to flow off. Ten ml of the 25 ml watery soil extract (see B) were reduced to about 0.5 ml by evaporation at room temperature *in vacuo*. Spots of this concentrated extract and of known solutions of dinitro phenol compounds were brought up from a 12.4 μ l capillar at a mutual distance of 3 cm. The solvent system used was *n*-butanol : chloroform = 1 : 1, equilibrated against water. After equilibration of the sheet in the tank for 7 h at 22°C, the solvent flowed over during the night. The sheet was dried in an air flow and next scanned for absorption of ultraviolet radiation. This detection method was about as sensitive as that on spot colour. The R_F values of DNOC, dinitro phenol (DNP) and 3,5-dinitro salicylic acid were 0.45, 0.29, and 0.24 respectively.

D. Spectrophotometry

Spectrophotometric determinations were carried out using an Unicam SP 600 spectrophotometer. DNOC was measured according to the method of Parker ¹¹, solved in ethyl methyl ketone, at 432 m μ .

RESULTS

The effect of the addition of DNOC to sterilized and non-sterilized soil on the emergence from afterwards sown cress seed is shown in Figure 1. Since the two graphs are from the same experiment, they are mutually comparable.

In non-sterilized soil, treated with 10 mg of Na-DNOC per pot, the emergence was strongly inhibited at first, but after a week the emergence percentage reached and surpassed that of the control. With 5 mg of Na-DNOC per pot, the enhancing effect of the treatment appeared from the beginning.

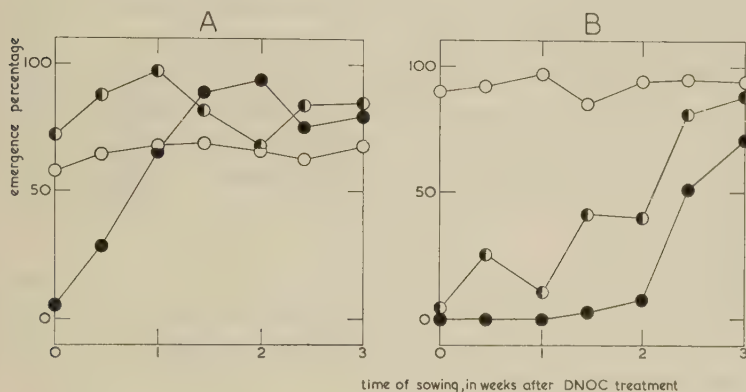


Fig. 1. The ultimate emergence percentage from cress seeds, sown at intervals after the addition of DNOC to A: non-sterilized, and B: sterilized soil.

Determination of the emergence percentage 5 days after sowing.

Symbols: ○ 0 mg of Na-DNOC per pot
 ◐ 5 " " " " "
 ● 10 " " " " "

The emergence percentage in sterilized soil, on the contrary, was never increased above the blank level by a preceding addition of DNOC, since this level was very high already. Moreover, the inhibition was much heavier and lasted for weeks, both with 5 mg and with 10 mg of Na-DNOC per pot.

In the control pots, emergence was always completed within 2 days. The addition of DNOC retarded the rate of emergence. In non-sterilized soil, this delay faded away within $1\frac{1}{2}$ and 2 weeks with 5 mg and 10 mg of Na-DNOC per pot respectively, but in sterilized soil the retardation due to 5 mg of Na-DNOC per pot disappeared after 3 weeks only.

The recovery from the inhibition both in percentage and in rate of emergence, appeared much quicker in non-sterilized than in sterilized soil. This suggests an at least partly microbial breakdown of DNOC, gradually admitting a better emergence. That in non-sterilized soil this recovered emergence exceeds that of the control

and even equals that of the sterilized control, may be due to three circumstances:

1. In contrast with the inhibitory effect of relatively large amounts of DNOC, a remaining, minute DNOC concentration might stimulate emergence.

2. Instead of by DNOC, such a stimulating effect could be exerted by one of the breakdown products of DNOC.

3. A comparison between the controls in Figure 1*A* and *B* shows that soil sterilization enhances the emergence percentage. Therefore, DNOC might partly sterilize the soil and, after having been removed for the greater part by the remainder of the microflora, leave a milieu that still preserves some of the advantages of the sterilization.

These three possibilities: stimulation of emergence by minute amounts of DNOC or by some decomposition product of DNOC, or an enhancing effect due to the sterilizing property of DNOC, are investigated below.

According to Figure 1*B*, DNOC breakdown in sterilized soil proceeds only slowly. For this reason, doses of Na-DNOC, varying from 0.01 mg to 50 mg per pot, were added to sterilized soil, the sowing followed closely and the course of the emergence was determined to see whether small amounts of DNOC were able to enhance the ultimate emergence percentage. In this series of experiments, the average percentage of ultimate emergence of the controls was rather low, *viz* 67. If, therefore, such an increasing effect occurred, it should be perceived. Figure 2 demonstrates, however, that DNOC acts as quite an ordinary poison only. There is no indication of a stimulatory effect at concentrations at which the inhibitory effect becomes no longer perceptible: when the curve comes up to 100 per cent of the control value, it flattens out. Measurements of the lengths of root and shoot of the seedlings led to the same result: DNOC either inhibited growth – from 0.1 mg of Na-DNOC per pot – or had no effect at all. Examination of the seeds at the end of the experiments confirmed the observation of Riepma¹⁵ that DNOC does not inhibit germination but prevents the radicle to grow out.

If some breakdown product of DNOC, *e.g.* some phenol derivative, should be responsible for the better emergence of seedlings

in DNOC-treated soil, then this compound should be extractable from the soil at the point of time that the stimulation of emergence appears. The extract should be able to enhance the emergence in non-treated soil and the concerning compound should be traced. From this suggestion the following type of experiment originated.

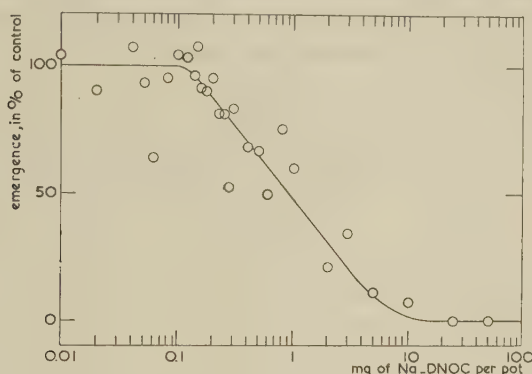


Fig. 2. The effect of the DNOC content of sterilized soil on the ultimate emergence of cress seedlings.

Mean values of data from up to 6 experiments.

To non-sterilized soil, 0 mg (control series), 5 mg (series A) or 10 mg (series B) of Na-DNOC per pot were added. After one week, a number of pots of the control series and of series A were extracted with ethanol (see Methods B), whilst other pots of these series were sown to check the appearance of the increase in emergence percentage. In the same manner, pots of the control series and of series B were manipulated another week later. In both cases, a definitely raised emergence percentage in the DNOC-treated soil was observed (Table 1). The extracts from the series A and B were added to non-sterilized soil again to give the series C and D respectively, which were sown at once. As can be concluded from Table 1, the extracts were unable to transmit the emergence-enhancing factor to fresh soil samples.

The extracts were analysed for their phenolic constituents. This led to the same result for the two extracts. Examination by paper chromatography revealed a yellow spot, travelling with the R_F -value of DNOC; it was the only UV-absorbing spot on the chromatogram.

Spectrophotometric analysis of the ethyl methyl ketone phase after shaking the extract with ethyl methyl ketone in a separation funnel, produced peaks at 375 $m\mu$ and 430 $m\mu$, with a minimum at 397 $m\mu$, which is characteristic for the DNOC ion in ethyl methyl ketone. In the extract from series A, 0.24 mg of Na-DNOC per pot could be determined, in the extract from series B 1.5 mg per pot. Addition of these quantities per pot to non-sterilized soil has no effect on the ultimate emergence percentage.

TABLE 1

The ultimate emergence percentage as the average value from 3 determinations, 5 days after sowing				
Series	Addition per pot	Days between addition and sowing	Ultimate emergence percentage	Increase over control
Control A	0 mg Na-DNOC	7	70	+ 24
	5 „ „		94	
Control B	0 „ „	14	73	+ 16
	10 „ „		89	
Control C	Extract control	0	76	— 2
	„ A		74	
Control D	„ control	0	71	0
	„ B		71	

Similar results were obtained in an experiment, in which the extraction was performed with water: the extracts could not induce an enhanced ultimate emergence percentage; their only detectable phenolic constituent was again DNOC.

It must be concluded that the increase in ultimate emergence percentage after DNOC treatment of non-sterilized soil is not caused by any ethanol- or water-extractable substance.

DISCUSSION

The enhanced emergence percentage in DNOC-treated soil is not due to a stimulation of *e.g.* germination by DNOC itself since, in all concentrations used, DNOC was either inhibitive or indifferent.

A soil bacterium capable of attacking DNOC was found by Gundersen and Jensen⁶; the authors suggest the first degradation step to be the removal of the para-nitro group. Smith *et al.*¹⁷ and Kikal and Smith⁹ showed the excretion of amino nitro phenols, acetamido nitro phenols, amino nitro salicylic acid, and

glycosides and other esters of these compounds, by mammals and insects poisoned with DNOC. In DNOC-treated soil, however, these or other phenolic breakdown products do not accumulate. The DNOC molecule being once attacked, its benzene ring must be ruptured comparatively rapidly, since no UV-absorbing substance could be traced in the soil extracts, except DNOC itself.

According to Dagley and Stopher² and Fernley and Evans⁴, soil micro-organisms may be able to decompose phenolic compounds into derivatives of the aliphatic muconic acid. Whatever non-phenolic breakdown product should accumulate, however, the ultimate emergence percentage is not increased by it, since no substance with such a property could be extracted from the soil with ethanol or water.

Whereas neither DNOC nor some breakdown product is likely to enhance the ultimate emergence percentage from seeds in non-sterilized soil, it is conceivable that the sterilizing property of DNOC does so. On the one hand, the emergence is far more complete in sterilized than in non-sterilized soil (cf. the control levels in Fig. 1*A* and *B*). On the other hand, DNOC, like DNP, stimulates respiration by uncoupling of the oxidative phosphorylation^{10 12} and, therefore, inhibits assimilatory processes^{5 7} in a wide variety of organisms, including micro-organisms¹. Jensen and Petersen⁸, testing 20 bacterial species on their resistance against DNOC, found values for the bacteriostatic concentration from $2 \cdot 10^{-4} M$ to $10^{-2} M$ of DNOC. The present author occasionally observed, in an experiment in which a *Pythium* infection appeared, that pots containing over 1 mg of Na-DNOC remained free from the disease.

It is suggested that the high DNOC concentrations used, 5 mg and 10 mg of Na-DNOC per pot, destroy part of the microflora in the soil, including micro-organisms which are harmful to germinated seeds. The remainder of the microflora contains micro-organisms which are able to decompose DNOC into indifferent end products. During this gradual detoxification the conditions for emergence grow more favourable and the emergence percentage rises as more time elapses between the DNOC treatment and the sowing. When the DNOC content is no longer toxic to the seedling, then, because of a changed composition in the microflora, a larger part of the germinated seeds will succeed in producing an emerging seedling than in non-treated soil.

It is an agricultural experience that spraying with DNOC preparations against weeds in cereal and leguminous crops often result in distinct increases in yield. Riepma¹⁴ reports an increase of 10 per cent from parcels which were free of weeds already; this rise should not be due to nitrogen manuring. The stimulation of root growth by DNOC solutions with flax seedlings in petri dishes, led Riepma¹⁵ to the suggestion that the yield increase might be caused by a stimulation of root growth. However, in spite of many efforts, this experiment on flax seedlings was found to be irreproducible. As with the emergence of cress seedlings in sterilized soil (Figure 2), DNOC – and dinitro-*sec*-butyl phenol (DNBP) too – was indifferent at the lower concentrations and toxic at the higher ones to root growth of flax seedlings.

Recently, Riepma¹⁶ reported that growth of plants on culture solutions was invariably inhibited by $5 \cdot 10^{-6} M$ to $10^{-4} M$ of DNOC concentrations.

The fact that DNOC is able to stimulate plant development in field experiments and in laboratory tests with non-sterilized soil but fails to exert any stimulating effect when added under more or less sterile conditions, together with the circumstance that it is – in all probability – not transformed into some other stimulating compound, indicates that DNOC in soil acts by its toxic properties mainly. On the one hand, it will partly sterilize the soil, since it is usually sprayed in an about $0.05 M$ solution. Whilst, on the other hand, it might temporarily reduce root growth. Yield increase by action of DNOC in soil can, therefore, result from sterilization and from growth retardation. The latter condition allows for a larger lapse of time before the young shoot has reached the size at which its differentiation stops. Therefore more leaf and kernel primordia may be developed, resulting in more leaves and larger ears on the stalk. Both more and larger leaves^{3 13} and more kernels per ear¹³ are found to be the result of DNOC spraying.

Besides by way of the soil, DNOC might stimulate plant development by action from the leaf surface. A discussion of this possibility, however, falls out of the scope of the present paper.

SUMMARY

The addition of initially poisonous amounts of the herbicide 4,6-dinitro-*o*-cresol (DNOC) to non-sterilized soil enhances the ultimate emergence

percentage from cress seeds, if these are sown some time after the DNOC treatment of the soil.

DNOC is removed from soil mainly by microbial activities.

The increase in the emergence percentage cannot be ascribed to a stimulation, exerted either by DNOC in any concentration or by some breakdown product of DNOC.

The ultimate emergence percentage of cress seedlings in sterilized soil surpasses that in non-sterilized soil.

It is suggested that the more complete emergence after DNOC addition to non-sterilized soil is due to a partial sterilization of the soil by DNOC, advantages of which remain preserved after removal of the DNOC by the remainder of the microflora.

The consequences of this view for an explanation of the yield-increasing effect of DNOC spraying in agriculture are discussed.

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REFERENCES

- 1 Blackman, G. E., Templeman, W. G. and Halliday, D. J., Herbicides and selective phytotoxicity. *Ann. Rev. Plant Physiol.* **2**, 199-230 (1951).
- 2 Dagley, S. and Stopher, D. A., A new mode of fission of the benzene nucleus by bacteria. *Biochem. J.* **73**, 16 pp. (1959).
- 3 Dobben, W. H. van, De invloed van DNC-besputtingen op de groeiwijze van winterrogge (with summary). *Verslag Centr. Inst. Landbouwk. Onderz. Wageningen* **1955**, 133-141 (1956).
- 4 Fernley, H. N. and Evans, W. C., Metabolism of 2 : 4-dichloro-phenoxy acetic acid by soil *Pseudomonas*. *Biochem. J.* **73**, 22 pp. (1959).
- 5 French, R. C. and Beevers, H., Respiratory and growth responses induced by growth regulators and allied compounds. *Am. J. Botany* **40**, 660-666 (1953).
- 6 Gundersen, K. and Jensen, H. L., A soil bacterium decomposing organic nitro-compounds. *Acta Agr. Scand.* **6**, 100-114 (1956).
- 7 Humphreys, T. E. and Dugger, W. M., Effect of 2,4-D and DNP on the uptake and metabolism of oxygenous substrates by corn roots. *Plant Physiol.* **34**, 112-116 (1959).
- 8 Jensen, H. L. and Petersen, H. I., Decomposition of hormone herbicides by bacteria. *Acta Agr. Scand.* **2**, 215-231 (1952).
- 9 Kikal, T. and Smith, J. N., Comparative detoxication. 6. The metabolism of 6-amino-4-nitro-*o*-cresol and DNOC in locusts. *Biochem. J.* **71**, 48-54 (1959).
- 10 Lott, J. R. and Rosene, H. F., Effects of 4,6-dinitro-*o*-cresol on water influx and oxygen uptake of excised onion roots. *Am. J. Botany* **43**, 69-72 (1956).

- 11 Parker, V. H., A method for the routine estimation of 3:5-dinitro-*o*-cresol (DNOC). The Analyst **74**, 646-647 (1949).
- 12 Parker, V. H., The effect of 3 : 5-dinitro-*o*-cresol on phosphocreatine and the adenosine phosphate compounds of rat tissues. Biochem. J. **57**, 381-386 (1954).
- 13 Riepma, P. Kzn., De reactie van granen op herbiciden in afhankelijkheid van het groeistadium (with summary). Gestencilde Mededel. Centr. Inst. Landbouwk. Onderz. Wageningen no. **11** (1953).
- 14 Riepma, P. Kzn., De reactie van granen en peulvruchten op kleurstoffen (with summary). Mededel. Landbouwhogeschool en Opzoekingsstas. Staat Gent **19**, 451-456 (1954).
- 15 Riepma, P. Kzn., De werking van DNC via de grond. Mededel. Landbouwhogeschool en Opzoekingsstas. Staat Gent **21**, 619-629 (1956).
- 16 Riepma, P. Kzn., Watergebruik, zoutopname en groei van planten onder invloed van DNC in de voedingsoplossing. Mededel. Landbouwhogeschool en Opzoekingsstas. Staat Gent **23**, 950-958 (1958).
- 17 Smith, J. N., Smithies, R. H. and Williams, R. T., Studies in detoxication. 48. Urinary metabolites of 4,6-dinitro-*o*-cresol in the rabbit. Biochem. J. **54**, 225-230 (1953).

INTERRELATIONSHIPS AMONG IRON, MANGANESE, AND MOLYBDENUM IN THE GROWTH AND NUTRITION OF TOMATOES GROWN IN CULTURE SOLUTION*

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INTRODUCTION

Considerable attention has been given over the past few years to the relationship between molybdenum and manganese in the growth of plants. Millikan's¹⁰ discovery of an antagonistic relationship between these elements was later corroborated by the results of Plant¹² and by Anderson and Arnot¹. An opposite relationship has been reported by Hewitt^{6 7} and Warington¹⁴. Later work by Warington¹⁵ showed the influence of iron supply on the manganese-molybdenum relationship. Recently, Candela and Hewitt² found that the concentrations of manganese and molybdenum in the growth medium influenced the nature of their interaction effects on plant growth and nutrition.

In view of the experimental evidence to date and the apparent discrepancies in the literature, it appeared logical to attempt further clarification of the nature of the iron-manganese-molybdenum relationship with a study designed to determine the influence of several different levels of these three variables on plant growth

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and composition. It was for this purpose that the present study was initiated.

EXPERIMENTAL METHOD

The experiment was carried out under greenhouse conditions using Marglobe tomatoes as the indicator crop. Seeds were germinated on a layer of cheesecloth stretched over the tops of paraffined 2-quart containers. The containers were filled with one-twentieth strength basic nutrient solution (Table 1) so that the cloth holding the seed was kept continually moist. At the appearance of the first true leaves, plants were transferred to larger containers for growth. Five uniform plants were grown in opaque ten-liter polyethylene containers fitted with polyethylene lids in which half-inch holes had been bored to hold the plants. Aeration was provided each container. Greenhouse temperatures were thermostatically controlled at $75 \pm 5^\circ\text{F}$ during the day and $55 \pm 5^\circ\text{F}$ at night. Supplementary light at 300 to 400 foot-candles was provided to give 16 hours of light followed by 8 hours of darkness.

TABLE 1

Sources of nutrients and composition of basic nutrient solution used for all treatments							
Source	Final concentration (me per liter of nutrient solution)						
	Ca	Mg	K	N	P	S	Cl
$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	8			8			
KH_2PO_4			1		1		
KNO_3			2	2			
K_2SO_4			2			2	
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$		2				2	
KCl			0.05				0.05
	(ppm of element)						
	B		Zn		Cu		
	0.27		0.13		0.03		
H_3BO_3							
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$							
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$							

Ingredients and composition of the basic nutrient solution are given in Table 1. Reagent-grade chemicals were used to prepare stock solutions which were further purified to remove molybdenum according to the method of Hewitt⁸ except that copper, zinc, iron, manganese, and molybdenum solutions were not further purified. Deionized water, obtained by passing tap water through ion-exchange resins, was used throughout the study. Resins were changed when the specific conductance of the columned water fell below 1×10^{-6} millimhos. Chemical analysis of the residue obtained after evaporating eight liters of water giving this reading showed no measurable amount of molybdenum present.

The basic nutrient solution in the individual containers was changed weekly, at which time the required amounts of manganese and molybdenum (Table 2) were also supplied. Iron was supplied in treatment-level amounts twice weekly. The pH of the nutrient solutions was not controlled and fluctuated depending on the level of iron supplied and the growth of the plants. However, at no time during the experiment was the pH of the nutrient solutions determined to be lower than 4.0 or higher than 6.8. Fluctuations in pH within these limits were preferred to the addition of differential amounts of acid or base as uncontrolled variables.

TABLE 2

Treatment variables *, treatment levels, and final concentration in the nutrient solution				
Treatment level No.	Coded treatment level (log scale)	Final concentration (ppm)		
		Fe	Mn	Mo
1	-1.68	0.001	0.001	0.000065
2	-1	0.005	0.005	0.0005
3	0	0.05	0.05	0.01
4	1	0.5	0.5	0.2
5	1.68	2.39	2.39	1.53

* Sources of variables were $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (Fe), $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ (Mn), and Na_2MoO_4 (Mo).

The plants were allowed to grow for a period of 36 days after which they were harvested and roots, stems, petioles, and leaves were separated. The plant material was washed briefly in deionized water to remove surface contaminants and dried in a forced-air oven at 65°C. After weighing, the material was ground in a small Wiley Mill prior to chemical analysis for manganese and molybdenum.

Following wet digestion of the ground plant material with nitric, sulfuric, and perchloric acids ⁴, samples of the various plant parts were analyzed for molybdenum ⁹ and manganese ¹³. A Beckman Model DU spectrophotometer with photomultiplier attachment was used to determine color intensities in the extracts. Because of the difficulties in obtaining reliable and meaningful data, Fe contents were not determined.

Experimental design

Solutions of different concentrations of iron, manganese, and molybdenum (Table 3) were imposed as treatment variables in a rotatable design described by Cochran and Cox ³. The general ranges of concentration of the variables were selected on the basis of information in the literature and on the basis of a preliminary experiment so as to obtain a range from trace amounts to toxicity. The final concentrations of the different levels were then selected so that they had a definite relationship to each other as indicated by the coded treatment levels in Table 2. The relationship be-

tween the actual concentration of Fe or Mn and the coded level (x) is expressed as

$$\text{Log}_{10} \text{Conc.} = x - 1.30103.$$

Similarly, the relationship between the actual concentration of Mo and the coded level (x) is given by

$$\text{Log}_{10} \text{Conc.} = 1.30103x - 2.0.$$

This was done to conform to the experimental design and to facilitate statistical analyses of the data. All treatments were randomized in each of three replications.

RESULTS AND DISCUSSION

Effect of treatment on yield

It was assumed that yield response to added iron, manganese, and molybdenum could be approximated by a second-order polynomial equation of the following type:

$$Y = b_0x_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2$$

where:

Y = the predicted yield:

b_1, b_2, b_3 = regression coefficients for linear effects of the variables iron, manganese, and molybdenum, respectively;

b_{12}, b_{13}, b_{23} = regression coefficients for interaction effects between variables;

b_{11}, b_{22}, b_{33} = regression coefficients for quadratic effects of variables;

x_1, x_2, x_3 = treatment levels of iron, manganese, and molybdenum respectively between the ranges of coded treatment levels $x = -1.68$ to $x = 1.68$.

Estimates of the b 's in this equation were obtained using observed yield data. These estimates, along with different combinations of variables, represented by the x 's in the equation, were then used to predict yields for various treatment level combinations of iron, manganese, and molybdenum according to the method of Hader *et al.*⁵ In the solution of the regression equation (Table 3) coded values for treatment levels (Table 2) were substituted for the terms of Fe, Mn, and Mo.

TABLE 3

Observed and predicted dry-weight yields (grams per pot) of tomato plants grown in solutions containing various treatment level combinations of iron, manganese, and molybdenum				
Treatment-level combination			Yield	
Fe	Mn	Mo	Observed (mean of 3 reps.)	Predicted (Y)*
2	2	2	10.94	12.63
2	2	4	6.67	8.11
2	4	4	2.34	6.61
2	4	2	9.69	10.41
4	2	2	0.48	-1.07
4	2	4	0.70	2.09
4	4	2	14.42	15.07
4	4	4	17.90	18.31
3	3	3	14.18	14.71
1	3	3	6.86	3.43
5	3	3	1.81	2.28
3	1	3	1.78	1.04
3	5	3	14.98	12.80
3	3	1	18.48	18.61
3	3	5	20.61	17.53
Analysis of variance of yield of tomato plants				
Source		d.f.	Mean square	F
Linear		3	169.97	63.90 **
Quadratic		3	139.57	52.47 **
Interaction		3	197.91	74.40 **
Lack of fit		5	30.33	11.40 **
Error		28	2.66	

* $Y = 14.71 - 0.34 \text{ Fe} + 3.50 \text{ Mn} - 0.32 \text{ Mo} - 4.20 \text{ Fe}^2 - 2.76 \text{ Mn}^2 + 1.19 \text{ Mo}^2 + 4.59 \text{ FeMn} + 1.92 \text{ FeMo} + 0.02 \text{ MnMo}$.

Where: Fe, Mn, and Mo refer to coded levels of these variables.

** Significant at 1% probability level.

The data of Table 3 shows a comparison of observed and predicted yields. The lack-of-fit term shown in the analysis of variance was significant and indicates some degree of failure of the equation to predict yields. This effect was small, however, in comparison with the linear, quadratic, and interaction effects as indicated by the size of the mean square values. Examination of the observed and predicted yields shows that agreement was generally good with respect to trends and orders of magnitude and for this purpose the equation should be satisfactory.

In Table 4 are shown the regression coefficients for linear, quadratic, and interaction effects. These coefficients appear as

TABLE 4

Regression coefficients (<i>b</i>) and their standard errors (<i>s_b</i>) for yield of tomato plants (grams dry-weight per pot)			
Coefficient	<i>b</i>	Standard error (<i>s_b</i>)	Mean square
Fe <i>b</i> ₁	-0.34	±0.26	4.74
Mn <i>b</i> ₂	3.50	±0.26	501.05 **
Mo <i>b</i> ₃	-0.32	±0.26	4.12
Fe × Mn <i>b</i> ₁₂	4.59	±0.33	505.64 **
Fe × Mo <i>b</i> ₁₃	1.92	±0.33	88.09 **
Mn × Mo <i>b</i> ₂₃	0.02	±0.33	0.01
Fe ² <i>b</i> ₁₁	-4.20	±0.38	319.49 **
Mn ² <i>b</i> ₂₂	-2.76	±0.38	138.59 **
Mo ² <i>b</i> ₃₃	1.19	±0.38	25.78 **

** Significant at 1% probability level.

numerical values in the prediction equation shown in Table 3. These regression coefficients are informative as to the effects of and relationships among variables. The linear effect of manganese was greater than that of iron or molybdenum. All three variables produced marked quadratic effects. Of particular interest, however, are the interaction components. Those for Fe × Mn and for Fe × Mo were much larger than that for Mn × Mo. It is the Mn × Mo relationship that some investigators have given particular attention to in the past. The data of Table 4 would suggest that the manganese relationships were exhibited through iron and the Fe × Mn and Fe × Mo interactions.

Response surfaces and interactions between iron, molybdenum and manganese

The predicted yields may be visualized with the aid of response surfaces which illustrate the effect of various treatment-level combinations in three-dimensional form (Figures 1 through 5). The surfaces shown are for various treatment level combinations of iron and molybdenum at different treatment levels of manganese. These figures are plotted in such a way that the independent variables (Fe and Mo) are linear on a scale of log₁₀. This accentuates the effect of these variables and permits an easier interpretation than would have been possible if they had been plotted arithmetically. Yield response to iron is indicated by heavy lines while yield response to molybdenum is shown by the light lines.

The effects of iron on phosphorus nutrition frequently complicate the interpretation of results of culture-solution studies. This is especially true when, as in this investigation, iron levels were varied over a wide concentration range while the phosphorus level was held constant. In such cases it is important to recognize that iron effects may manifest themselves through phosphorus nutrition. Nevertheless, unless the experiment is designed to measure these effects on phosphorus nutrition, it is not possible to distinguish between direct and indirect effects of iron treatment and interpretations of results must necessarily be restricted to the effects of iron additions *per se*. The observed phenomena were still due to iron additions regardless of whether the effects are direct or indirect. Thus, in the subsequent discussion it should be kept in mind that while responses to different levels of applied iron are being discussed, indirect responses to differences in phosphorus nutrition may be involved as well.

The interaction between iron and manganese was quite marked, as indicated by the mean square value for $\text{Fe} \times \text{Mn}$ shown in Table 4. This was manifested as an ameliorating effect of manganese on iron toxicity and is evident from inspection of the surfaces. In Figure 1 it is seen that yield response to added iron (heavy lines) began to decrease markedly between Fe_2 and Fe_3 and that zero yields were predicted between Fe_3 and Fe_4 at all molybdenum levels. However, as the manganese supply was increased (Figures 2, 3, 4, and 5), predicted yield response to added iron also increased; that is, yields became correspondingly higher at higher iron levels. This resulted in a general shifting or tilting of the response surface along its horizontal axis until the general slope of the iron-response curves (heavy lines) was reversed.

A marked interaction effect between iron and molybdenum also occurred, as shown by the mean square value for $\text{Fe} \times \text{Mo}$ in Table 4. An examination of the curves for yield response to molybdenum (light lines) shows that when molybdenum was added at low levels of iron, yields were depressed. Addition of molybdenum at high levels of Fe, however, resulted in general increases in yield. At the intermediate levels of iron, yields were at first decreased, reached a minimum, and then increased as iron was added (Figures 1 through 5). This caused the entire response surface to appear as a saddle. This saddle effect can be explained as a geometrical

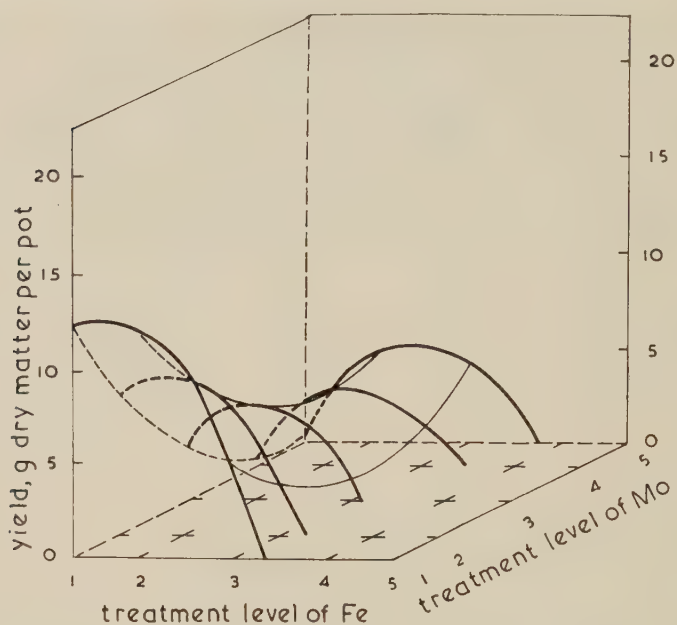


Fig. 1. Response surface for treatment level combinations of iron and molybdenum at Mn₁.

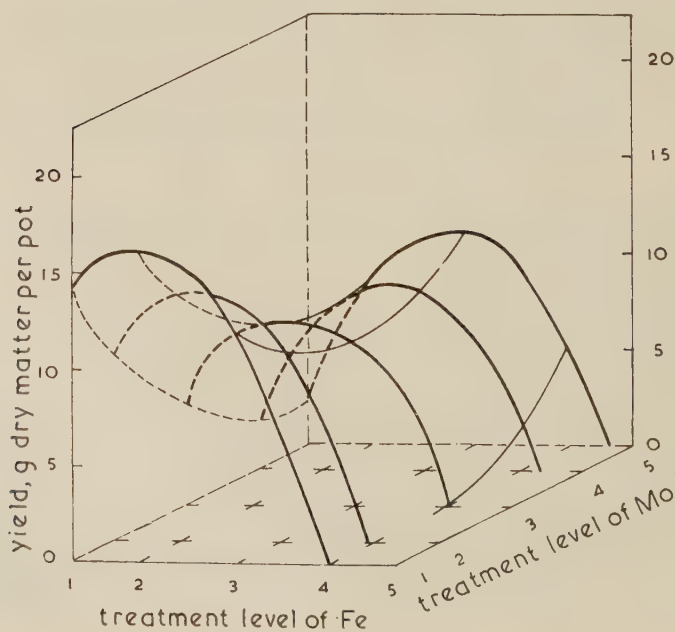


Fig. 2. Response surface for treatment level combinations of iron and molybdenum at Mn₂.

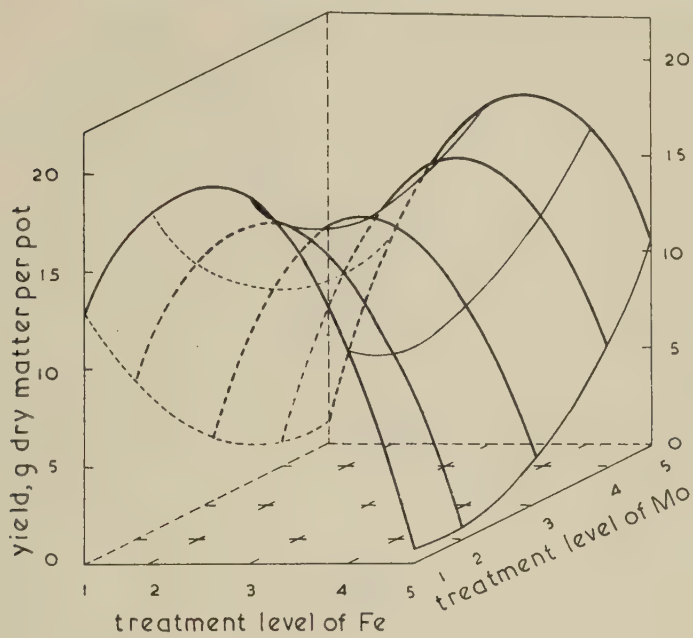


Fig. 3. Response surface for treatment level combinations of iron and molybdenum at Mn₃.

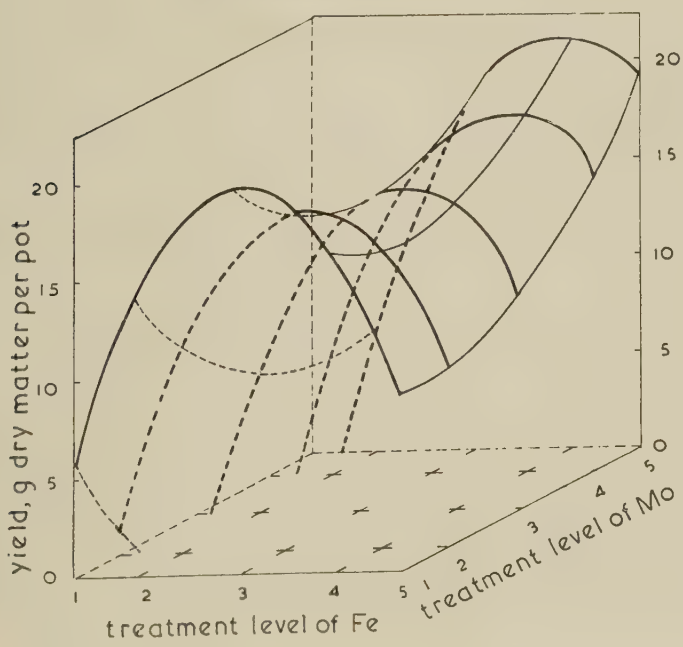


Fig. 4. Response surface for treatment level combinations of iron and molybdenum at Mn₄.

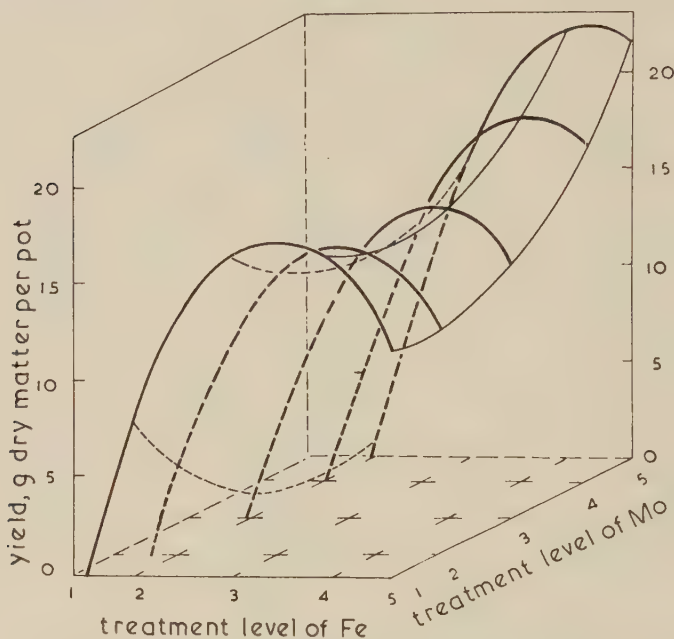


Fig. 5. Response surface for treatment level combinations of iron and molybdenum at Mn_5 .

manifestation of interaction effects of the variables on growth. An examination of the response surfaces for different levels of manganese (Figures 1 through 5) shows that at *low* levels of iron (Fe_1 and Fe_2) increasing the molybdenum supply (along the light lines) *decreased* the yields; however, at *high* levels of iron (Fe_4 and Fe_5) molybdenum additions increased the yields. Thus, there was a differential yield response to molybdenum which was dependent on the level of iron supplied. Since opposite yield trends occurred at the low and high iron levels, the intermediate levels showed the effect of both of these trends, resulting in the saddle effect. This differential response to added molybdenum is illustrated most clearly in Figure 4, where the entire response surface over the concentration ranges for which the prediction equation was derived is visible and where the negative slope of the molybdenum-response curve at Fe_1 and the positive slope of the molybdenum-response curve at Fe_5 are quite evident. This differential type of response was also observed in an experiment preliminary to the one reported here.

Further evidence of the iron-molybdenum interaction was evident from the shifting points of maximum yield on the iron-response curves as the molybdenum supply was increased. When manganese was kept at the Mn_1 level (Figure 1), for example, it was found that maximum predicted yields occurred at $Fe_{1.5}$ for Mo_1 , at $Fe_{1.7}$ for Mo_2 , at Fe_2 for Mo_3 , at $Fe_{2.2}$ for Mo_4 , and at $Fe_{2.4}$ for Mo_5 . The same general trend existed at each of the other manganese levels. This indicates that as the molybdenum supply was increased (along the light lines), higher levels of iron were required to obtain maximum yields at all levels of manganese.

There was also some evidence of a three way interaction between iron, manganese, and molybdenum. It was shown that there was a shifting of the points of maximum yield on the iron-response curves as the molybdenum supply was increased. However, the iron levels corresponding to maximum yield were higher as manganese was increased. Also, the saddle effect previously discussed was especially noticeable at lower manganese levels (Figures 1 and 2) and tended to become less pronounced as the manganese supply was increased (Figures 3, 4, and 5). Further examination of Figures 1 through 5 shows evidence of this three-way iron $\times$ manganese $\times$ molybdenum interaction: -

1. At levels of iron and molybdenum corresponding to Fe_1 , Fe_2 , Fe_3 , and Mo_1 , Mo_2 , and Mo_3 , respectively, yield increased, reached a maximum, and then decreased as manganese was added. The level of manganese at which the maximum was reached varied with the iron and molybdenum levels.
2. At levels of iron and molybdenum corresponding to Fe_4 , Fe_5 , and Mo_4 and Mo_5 respectively, yield response to manganese continued to increase as manganese itself was increased and no maximum was attained.

Thus, yield response to manganese depended not solely on the level of iron, as indicated by the significance of the iron $\times$ manganese interaction previously discussed, but on the levels of *both* iron and molybdenum. A statistical measurement and test of significance of this three-factor effect was not possible due to the limitations of the experimental design and second order quadratic equation. Its effects on yields are, however, quite evident from the data.

Millikan ¹⁰ and also Anderson and Arnot ¹ found that added molybdenum alleviated the toxic effects of excess manganese. The

data of this study show that this effect indeed occurred, but only at *high* iron levels. Hewitt^{6,7} and Warington¹⁴ reported that molybdenum accentuated manganese-induced iron chlorosis. The data of this study clearly show that this effect occurred also, but only at *low* iron levels. It would appear that these different investigators may have measured yield effects in different portions of what is actually, according to the results of the present study, a differential yield response curve and that their seemingly contradictory results were manifestations of differences in iron nutrition. Candela and Hewitt² found that the effects of increased manganese supply were injurious at low molybdenum levels but not at higher levels. The data of the present study show a similar effect although the manganese $\times$ molybdenum interaction as measured by the analysis of variance (Table 4) was not significant. Warington¹⁵ found that molybdenum additions counteracted the yield-depressing effect of excess iron. The results reported here are in agreement but show that this effect tended to be greater at higher manganese levels. It is worthy to note from studies reported here that the iron $\times$ molybdenum and the iron $\times$ manganese interactions were quite large in comparison with manganese $\times$ molybdenum interaction. It is suggested that the manganese-molybdenum relationships which have been reported were probably indirect effects which were manifested through the iron relationships.

Effect of treatment on absorption of molybdenum and manganese

The effects of treatments on yield were of primary interest in these investigations. Some information, however, on the effects of treatment on absorption of molybdenum and manganese were also obtained.

The effect of certain treatment-level combinations on yield was quite drastic, especially where low and/or high levels of iron and/or manganese were involved. It was necessary in some of these cases to composite samples of plant material from the three replications in order to obtain a sample large enough for chemical analyses. In the more extreme cases, there was not sufficient plant material to permit chemical analyses. Consequently, plant analysis data will be discussed from the standpoint of trends, and statistical interpretation will be based on analyses of variance where applicable.

Data for the effects of treatment on the uptake of molybdenum

Effect of treatment on the uptake and relative distribution of molybdenum

Molybdenum uptake by roots was reduced while uptake by leaves was generally increased as iron was added (Table 5). This was

TABLE 6

Uptake of manganese ($\mu\text{g}/\text{pot}$) by various plant parts and dry-weight yields of tomato plants grown in nutrient solution. Mean of 3 replications								
Treatment			Uptake by plant parts				Total uptake	Observed yield (whole plant) g per pot
Fe	Mn	Mo	Roots	Stems	Petioles	Leaves		
Effect of iron additions								
1	3	3	601.5	34.7	29.8	461.6	1127.6	6.86
3	3	3	349.5	98.2	47.1	454.6	949.4	14.18
2	4	2	7335.4	156.5	168.3	2476.9	10137.6	9.69
4	4	2	3663.4	165.6	85.6	1662.7	5577.2	14.42
2	4	4	2198.0	41.1	35.0	559.2	2834.0	2.34
4	4	4	4616.8	232.4	167.8	1974.6	6991.3	17.90
Effect of manganese additions								
3	1	3	37.8	4.5	6.6	8.8	57.6	1.78
3	3	3	349.5	98.2	47.1	454.6	949.4	14.18
3	5	3	25355.0	693.2	581.1	8410.9	36202.4	14.98
2	2	2	38.0	16.0	10.6	144.9	209.5	10.94
2	4	2	7335.4	156.5	168.3	2476.9	10137.6	9.69
2	2	4	26.7	14.1	28.8	130.5	200.0	6.67
2	4	4	2198.0	41.1	35.0	559.9	2834.0	2.34
Effect of molybdenum additions								
3	3	1	522.9	117.0	115.5	494.9	1250.4	18.48
3	3	3	349.5	98.2	47.1	454.6	949.4	14.18
3	3	5	204.6	58.8	44.3	580.3	887.9	20.61
2	2	2	38.0	16.0	10.6	144.9	209.5	10.94
2	2	4	26.7	14.1	28.8	130.5	200.0	6.67
2	4	2	7335.4	156.5	168.3	2476.9	10137.6	9.69
2	4	4	2198.0	41.1	35.0	559.2	2834.0	2.34
4	4	2	3663.4	165.6	85.6	1662.7	5577.2	14.42
4	4	4	4616.8	232.4	167.8	1974.6	6991.3	17.90
LSD for:						P =		
Total uptake						.05		.01
Plant parts for same treatment						1100.7		1565.6
Same plant parts for different treatments . .						1330.0		1785.8
						1223.6		1659.7

especially noticeable in the high-manganese-high-molybdenum treatments ($\text{Fe}_2\text{Mn}_4\text{Mo}_4$ *versus* $\text{Fe}_4\text{Mn}_4\text{Mo}_4$). This would seem to imply that additions of iron stimulated the translocation of molybdenum from roots to leaves. The data also show that total molybdenum uptake by the plant was reduced by iron additions, this effect again being most noticeable at higher molybdenum levels. Further evidence of this tendency is shown in Table 5 by the data for root uptake of molybdenum as molybdenum was added. These data show that when molybdenum was added at a lower iron level (Fe_2) the uptake of molybdenum by roots was increased by a factor of 381 at Mn_2 and a factor of 125 at Mn_4 and that these increases were accompanied by decreases in yield (compare $\text{Fe}_2\text{Mn}_2\text{Mo}_2$ *versus* $\text{Fe}_2\text{Mn}_2\text{Mo}_4$ and $\text{Fe}_2\text{Mn}_4\text{Mo}_2$ *versus* $\text{Fe}_2\text{Mn}_4\text{Mo}_4$). Yet when molybdenum was added at a higher iron level (Fe_4), root uptake of molybdenum was increased by a factor of only 40 (compare $\text{Fe}_4\text{Mn}_4\text{Mo}_2$ *versus* $\text{Fe}_4\text{Mn}_4\text{Mo}_4$) and yields increased. Similar trends were shown by the data for total uptake. It will be recalled from the discussion on yield that molybdenum additions at low iron levels resulted in decreases in yield; however, molybdenum additions at high iron levels resulted in increases in yield. It would appear from the data on molybdenum absorption that this interaction effect had its foundation in the effect of iron on molybdenum absorption and translocation.

Anderson and Arnot¹ reported that applied manganese reduced the uptake of molybdenum by white clover. Mulder¹¹ found that manganese additions reduced molybdenum uptake by cauliflower and lettuce grown on acid soils but that tomatoes did not respond similarly, either in soil or culture media. Candela and Hewitt² reported that manganese additions increased the molybdenum content of tomatoes grown in sand culture. The results of this study show an apparent *differential* effect of manganese on molybdenum uptake which depended on the iron level (compare $\text{Fe}_2\text{Mn}_2\text{Mo}_4$ *versus* $\text{Fe}_2\text{Mn}_4\text{Mo}_4$ and $\text{Fe}_3\text{Mn}_3\text{Mo}_3$ *versus* $\text{Fe}_3\text{Mn}_5\text{Mo}_3$). Thus, at a lower iron level manganese decreased molybdenum uptake while at a higher iron level manganese increased it. This latter effect tends to emphasize the antagonistic relationship between manganese and iron, iron having been shown to decrease molybdenum uptake.

Millikan¹⁰ and Plant¹² have reported a depressive effect of added molybdenum on manganese absorption. The results of this

study show that this effect indeed occurred, but only at the lower iron and higher manganese levels. It is significant to note that both Millikan and Plant were working with growth media high in manganese, thus the data of this study tend to support their results.

It would appear from the data of this study that manganese absorption was influenced by a three-way interaction among iron, manganese, and molybdenum, an interaction which has also been mentioned previously in connection with treatment effects on molybdenum absorption as well as yield.

SUMMARY AND CONCLUSIONS

In a study designed to determine the effects of different levels of iron, manganese, and molybdenum on the growth and nutrition of tomato plants, a solution-culture experiment was carried out in the greenhouse under relatively controlled conditions. Results indicated that:

1. Iron $\times$ molybdenum and iron $\times$ manganese interactions on yield were found to be much higher than that for manganese $\times$ molybdenum. It was suggested that the manganese relationship to molybdenum was exhibited indirectly through these other interactions. Yield data strongly suggested the existence of a three-way iron $\times$ manganese $\times$ molybdenum interaction.
2. Added iron counteracted the yield-depressing effect of added manganese, but the amount of iron required to attain maximum yield increased as the molybdenum supply was increased.
3. At lower iron levels, added molybdenum *decreased* yields; at higher iron levels, added molybdenum *increased* yields.
4. Iron additions appeared to stimulate the translocation of molybdenum from roots to leaves but reduced total molybdenum uptake. Added molybdenum tended to accumulate in roots in toxic quantities at lower iron levels but not at higher iron levels.
5. At both lower iron and lower manganese levels, added molybdenum had little effect on manganese uptake; at lower iron and higher manganese levels, molybdenum *decreased* the manganese uptake; at higher iron and higher manganese levels, molybdenum *increased* manganese uptake.

It was concluded that an iron-manganese-molybdenum relationship existed which governed growth response to any of these three elements and that the relationship of manganese to molybdenum was manifested indirectly through iron and the iron $\times$ manganese and iron $\times$ molybdenum interactions.

The results of this investigation illustrate the necessity of an adequate number of levels of treatment variables across sufficiently wide concentration ranges, if interrelationships are to be satisfactorily described.

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REFERENCES

- 1 Anderson, A. J. and Arnot, R. H., Fertilizer studies on basaltic red loam soil from the Linsmore district, New South Wales. *Australian J. Agr. Research* **4**, 29-43 (1953).
- 2 Candela, M. I. and Hewitt, E. J., Molybdenum as a plant nutrient: IX. The effects of different molybdenum and manganese supplies on yield and on the uptake and distribution of molybdenum in tomato plants growing in sand culture. *J. Hort. Sci.* **32**, 149-161 (1957).
- 3 Cochran, W. G. and Cox, G. M., *Experimental Designs*, 2nd Edition. John Wiley and Sons, New York (1957).
- 4 Evans, H. J., Purvis, E. R., and Bear, F. E., Colorimetric determination of molybdenum by means of nitric and perchloric acids. *Anal. Chem.* **22**, 1568-1569 (1950).
- 5 Hader, R. J., Harward, M. E., Mason, D. D. and Moore, D. P., An investigation of some of the relationships between copper, iron, and molybdenum in the growth and nutrition of lettuce: I. Experimental design and statistical methods for characterizing the response surface. *Soil Sci. Soc. Am. Proc.* **21**, 59-64 (1957).
- 6 Hewitt, E. J., Experiments on iron metabolism in plants: I. Some effects of metal-induced iron deficiency. *Ann. Rept. Long Ashton Research Sta.* 66-80 (1948).
- 7 Hewitt, E. J., Metal interrelationships in plant nutrition: II. The relation of metal toxicity, molybdenum, and nitrogen source to chlorophyll and magnesium content of beet in sand culture. *J. Exptl. Botany* **5**, 110-118 (1954).
- 8 Hewitt, E. J. and Jones, E. W., The production of molybdenum deficiency in plants and in sand culture, with special reference to tomato and Brassica crops. *J. Pomol. Hort. Sci.* **23**, 254-262 (1947).
- 9 Johnson, C. M. and Arkley, T. H., Determination of molybdenum in plant tissue. *Anal. Chem.* **26**, 572-573 (1954).
- 10 Millikan, C. R., Lower leaf scorch of flax. *J. Dept. Agr. Victoria* **46**, 511-517, 566-576 (1948); **47**, 37-41 (1949).
- 11 Mulder, E. G., Molybdenum in relation to growth of higher plants and micro-organisms. *Plant and Soil* **5**, 368-415 (1954).
- 12 Plant, W., Use of lime and sodium molybdate for the control of whiptail in broccoli. *Nature* **165**, 533-534 (1950).
- 13 Sherman, G. D., McHargue, J. S. and Hodgkiss, W. S., Determination of active manganese in soil. *Soil Sci.* **54**, 253-257 (1942).
- 14 Warington, K., Some interrelationships between manganese, molybdenum and vanadium in the nutrition of soybean, flax, and oats. *Ann. Appl. Biol.* **38**, 624-641 (1951).
- 15 Warington, K., The influence of iron supply on toxic effects of manganese, molybdenum, and vanadium on soybean, peas, and flax. *Ann. Appl. Biol.* **41**, 1-22 (1954).

POPULATIONS MICROBIENNES DES BOIS

IV. HUMIFICATION DE COPEAUX DANS LA NATURE

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INTRODUCTION

L'un de nous (F.M.) a consacré diverses études à l'écologie et à la systématique des champignons lignicoles et ceci devait nous conduire à envisager l'humification des bois. Le sujet a été peu étudié ** et pourtant les brindilles et branches mortes sont incorporées en quantités appréciables aux litières des forêts. D'autre part, le bois – débarrassé de son "écorce" – est un substrat relativement homogène, formé de cellules pour la plupart lignifiées, dépourvu au contraire de parenchymes et de réserves facilement attaquables. Il semblerait donc, *a priori*, permettre mieux que les pailles ou les racines, d'envisager le rôle si controversé de la lignine dans la formation des substances humiques. Mais les bois, même divisés, se décomposent lentement et sont peu favorables à l'expérimentation; pour la même raison leur emploi en agriculture comme amendements n'est guère entré dans la pratique. Bien mieux, nous connaissons des jardins dont les allées sont recouvertes de sciure pour empêcher le développement des mauvaises herbes.

Cependant, nous avons eu la chance de voir mettre à notre disposition de volumineux tas de copeaux entreposés sur le sol depuis un temps plus ou moins long – jusqu'à dix ans – sans remaniements ni apports de substance fraîche; de sorte qu'il nous a été possible, en un petit nombre d'années, de reconstituer la suite des processus d'humification.

* Avec la collaboration technique de Melle. M. Clet.

** Kononova, dans sa monographie⁵, signale de Troussov (1916) une étude que nous n'avons pas eu en mains.

MATÉRIEL ET MÉTHODES

La saboterie de Génicourt sur Meuse – dont nous remercions ici la direction – traite des grumes de hêtre et accessoirement de frêne et de peuplier en produisant par sciage longitudinal des sortes de copeaux, inutilisables comme combustible et qu'elle entasse, en bordure de la route nationale, sur les alluvions calcaires de la Meuse. Le tas, d'abord haut de 2 à 3 m, léger et aéré, diminue peu à peu de volume, devient plus compact et se mêle progressivement par la base au sol sous-jacent, grâce à l'action des taupes, vers de terre, etc.

Les pourritures des bois ont été largement étudiées et ceci nous a conduits à renoncer, dans le présent travail, aux méthodes inspirées des techniques de l'analyse végétale (Waksman). Il nous a semblé plus intéressant d'envisager les caractères des substances humiques formées et, pour ce faire, nous avons eu recours, suivant les conseils de Henin ², aux techniques pédologiques mieux adaptées à ce but particulier.

Les échantillons, prélevés en des points d'âges et d'aspects différents, sont soumis à une analyse microbiologique dont nous rendrons compte ultérieurement et à une analyse physico-chimique dans laquelle nous déterminons:

- 1) le pH, à l'électrode de verre, sur le produit simplement humecté d'eau distillée.
- 2) l'Azote total, selon Kjeldahl. (Titration acidimétrique de l' NH_3 distillé, en présence de H_3BO_3).
- 3) les cendres, par calcination au four à moufle.
- 4) les acides humiques et fulviques (extraction au NaF 1% selon la méthode Chaminade, et détermination par manganimétrie).
- 5) sur le résidu de l'extraction précédente, la lignine, par attaque à l'acide sulfurique concentré, à froid puis à chaud après dilution (Méthode De Forest).
- 6) la capacité d'échange par l'acétate d'ammonium, à pH 7,0.

Une description plus précise de nos techniques sera donnée ailleurs (Jacquin et Mangenot ⁴).

Les deux premiers chiffres fournissent des indications importantes du point de vue écologique: acidité ionique et teneur en N sont des facteurs essentiels, liés à la composition du peuplement microbien.

Quant à la fraction désignée, suivant l'usage, sous le nom de lignine, elle correspond, en partie, aux insolubles de la méthode Waksman. Si, dans le bois frais, elle est constituée principalement de lignine, elle comprend dans les produits altérés des substances de plus en plus modifiées et de nature mal définie, voire même toute différente. On remarquera cependant que les chiffres obtenus correspondent bien à ce que nous savons de la décomposition des bois: les valeurs les plus faibles se rencontrent en général dans les sciures pâles – ou bien dans des produits sombres mais profondément altérés – tout en se maintenant au voisinage de celles que nous trouvons dans le bois sain; il est probable que les autres constituants de la matière ligneuse sont dégradés en même temps que la lignine et nous trouvons fréquemment, associées

aux pourritures blanches, des bactéries cellulolytiques. Au contraire dans les cultures pures d'un *Trechispora* lignivore, commun sur nos copeaux, le taux de lignine s'abaisse sensiblement (Jacquin et Mangenot ⁴).

RESULTATS EXPERIMENTAUX

Les premiers prélèvements dont nous rendrons compte ont été effectués en Automne, dans un tas âgé de 2 à 3 ans et désigné ici par la lettre C. En surface, sur une épaisseur de 5 cm environ (C1), les copeaux sont encore parfaitement distincts, mais colorés en gris foncé à noir intense. Au-dessous, sur quelque 20 cm, le produit est sombre, brun-noir, plus humide (C2). Il recouvre un troisième horizon (C3) de 40 cm d'épaisseur environ où la fermentation est plus active et la température relativement élevée. Les copeaux y sont encore bien conservés, de teinte normale ou jaune-brun à brun. Enfin, à proximité du sol (C5), la structure est altérée, la substance transformée en une masse granuleuse brun-tabac. Vers les bords du tas, cette stratification est peu nette, le produit est hétérogène, tantôt clair, tantôt brun ou noir (C4 et C6). La population fongique est surtout représentée ici par les stades *Phialophora* et *Melanomma* de Mangenot ⁶ bien que, localement, apparaissent des foyers de pourriture blanche.

Un an plus tard, l'épaisseur du tas s'est réduite à quelque 30 cm. La surface s'est apparemment peu transformée: les copeaux y sont toujours visibles, gris foncé (C7, C8). Au-dessous s'étend une région de teinte pâle, blanc-jaunâtre, où les fragments tout en gardant leur forme sont agglomérés et devenus si fragiles qu'ils se réduisent en poussière par simple trituration entre les doigts (C9, C10). Cette zone repose sur un horizon organo-minéral, brun-tabac entremêlé de particules calcaires (C15). Cependant, dans certaines régions, ces deux dernières couches sont remplacées par un produit noir ou brun-noir, profondément dégradé et ressemblant à du terreau (C12-C14). A partir de ce moment, le tas se recouvre à la belle saison de *Convolvulus sepium* L., tandis qu'*Agropyrum repens* L. l'envahit par les bords. Les populations fongiques se sont enrichies du stade *Bisporomyces*; dans les zones noircies elles comportent en outre les stades *Melanomma* et *Mollisia*, auxquels se substitue ailleurs un stade lignivore (C9, C10) représenté principalement par *Trechispora* sp. mentionné plus haut.

Enfin au printemps suivant les régions décolorées se sont assom-

bries (C 11), suggérant l'installation, dans le bois pourri, de nouvelles espèces, mais nous n'avons pas pu, à ce moment, procéder à une analyse microbiologique.

Les résultats des analyses concernant le tas C sont consignés dans le Tableau 1.

TABLEAU 1

Composition chimique (% de matière sèche à 105°C) des copeaux, série C (2 à 5 ans)									
No.	Profondeur	Date	Teinte	pH	N	Acides humiques	Acides fulviques	Lignine	Cendres
C1	Surface	Nov. 1957	noire	6,30	0,46	—	—	—	—
C2	Moyen	„	brun-noir	6,10	0,35	—	—	—	—
C3	„	„	variable	5,15	0,21	—	—	—	—
C4	„ *	„	„	5,70	0,21	0,80	—	30	—
C5	Profond	„	brun foncé	6,80	0,27	—	—	—	—
C6	„	„	„	5,00	0,45	—	—	—	—
C7	Surface	Nov. 1958	noire	—	0,88	0,79	1,94	—	—
C8	„	Fév. 1959	„	7,1	0,66	0,86	3,18	28,3	—
C9	Moyen	Nov. 1958	pâle	—	0,38	3,27	2,73	—	—
C10	„	Fév. 1959	„	4,1	0,32	2,08	7,27	25,8	—
C11	„	Avr. 1959	brunâtre	4,85	0,92	6,22	5,72	28,4	12,66
C12	„	Nov. 1958	noire	—	1,16	0,73	0,80	—	—
C13	„	Fév. 1959	„	7,36	0,77	4,72	3,29	34,3	17,28
C14	„	Avr. 1959	„	7,00	1,15	2,68	3,33	—	15,79
C15	Sous C 11	„	tabac	7,80	0,86	4,40	3,28	36,0	29,18

* Capacité d'échange: 19,2 me/100 g.

Etant données les fluctuations appréciables enregistrées pour tous les échantillons d'un prélèvement à l'autre, le lecteur aura surtout intérêt à comparer les chiffres concernant des produits de types différents recueillis à la même date, par exemple C7, C9, C12 ou C8, C10, C13. Il faut noter cependant que C11 est un échantillon assez particulier dont nous discuterons plus loin la signification. Cette réserve faite, on pourra constater que, dans tous les cas où les comparaisons sont possibles, les produits pâles sont pauvres en N, renferment plus d'humus extractible par le NaF (acides humiques + acides fulviques) et moins de lignine que les produits sombres.

On peut rapprocher de ces chiffres les résultats obtenus en analysant les échantillons d'un autre tas de copeaux, marqué G, d'aspect analogue, d'âge voisin (4 à 5 ans) mais où les différentes zones sont moins nettement distinctes. Ces résultats, très fragmentaires, sont les suivants:

En Juillet 1956, deux prélèvements dans des régions brunies présentaient un pH de 6,2 et des teneurs en acides humiques et fulviques de 0,19-0,20% et 0,58-0,97% respectivement, tandis qu'un échantillon de sciure décolorée de pH 4,2 en renfermait 1,9 et 4,2%.

Un an plus tard on notait dans une zone moyenne, hétérogène et partiellement décolorée, de pH 5,0; N: 1,20%; Acides humiques: 2,8%; lignine: 34,5%; capacité d'échange: 43,4 me/100 g. Ailleurs on enregistrait:

G2, surface noircie	pH: 5,2	N: 0,56%
G3, zone moyenne hétérogène	4,0	0,62%
G4, „ profonde décolorée	4,9	1,15%
G5, „ „ noire	6,7	1,50%

Nous nous sommes adressés d'autre part à un dépôt sensiblement plus ancien, (marqué D) âgé de 8 à 9 ans et situé dans une prairie basse à proximité du canal latéral à la Meuse. Les copeaux y sont, la plupart du temps, méconnaissables et l'on croirait plutôt avoir affaire à un terreau d'un noir intense et chargé de particules calcaires. Cependant cette formation, épaisse d'une vingtaine de centimètres, renferme encore çà et là des débris de planchettes attestant son origine (D7, D8). Par ailleurs, en certains points, le produit est mieux conservé: ici les copeaux partiellement désagrégés ont pris une teinte brun-rougeâtre (D4, D5), là ils sont pâles et analogues à ceux que nous avons rencontrés plus haut en C9, C10 (D1 à D3) et ils portent, encore actuellement, *Pluteus cervinus* et *Corticium* sp.

L'ensemble de ce dépôt est progressivement envahi par la végétation: quand nous avons commencé de l'étudier, il était en grande partie nu; par la suite nous avons vu s'y installer, dans les régions les plus modifiées, *Calamagrostis epigeios* (L.) Roth. Enfin les zones non-envahies par les graminées portaient (relevé de Juin 1959) les espèces communes dans les terrains vagues de la région: *Equisetum arvense*, *Geranium robertianum*, *Pastinaca sativa*, *Galeopsis tetrahit*, *Galium aparine*, *Valerianella olitoria*, etc.

Quant au peuplement microbien, il comporte, comme précédemment, dans les régions noircies ou brunies (D4 à D8) des représentants des stades *Melanomma* et *Bisporomyces*, tandis que ce dernier fait pratiquement défaut dans les zones pâles où se développent au contraire les formes lignivores.

Les résultats concernant le tas D sont consignés dans le Tableau 2; dans ce dernier ne figurent pas d'indications de profondeur, le produit ne comportant pas de couches distinctes. Tout au plus

TABLEAU 2

Composition chimique (% de matière sèche à 105°C) des copeaux, série D (8 à 10 ans)								
No.	Date	Teinte	pH	N	Acides humiques	Acides fulviques	Lignine	Cendres
D 1	Nov. 1957	pâle *	6,30	0,22	1,35	—	24,7	—
D 2	Nov. 1958	„	7,1-8,1	0,77	1,62	3,65	27,8	—
D 3	Avr. 1959	„	5,45	0,37	2,32	9,6	—	2,3
D 4	Nov. 1958	brun-roux	—	0,32	1,59	1,88	—	—
D 5	Fév. 1959	„	4,6	0,66	2,50	2,95	38,1	—
D 6	Avr. 1959	± sombre	7,35	0,87	2,83	4,82	22,7	27,85
D 7	Nov. 1957	noire **	7,0	1,25	2,18	—	49,0	—
D 8	Avr. 1959	„	7,5	1,12	2,78	1,82	32,4	28,64

* Capacité d'échange: 14,5 me/100 g.

** „ „ : 72 me/100 g.

peut-on noter que les régions pâles reposent d'habitude sur un horizon organo-minéral sombre. Sous cette réserve, le Tableau 2 appelle les mêmes remarques et commentaires que le Tableau 1, le produit D6 étant sans doute homologue de C11.

CONCLUSION

Nous exposerons ultérieurement les conclusions générales que fournit l'ensemble de nos expériences et nous nous contenterons de noter ici les points suivants:

1) Au cours de son humification, la substance s'enrichit en azote. Le phénomène s'explique en partie par la diminution du poids total due à la minéralisation de la matière organique. Cependant l'enrichissement est faible, sans doute proportionnel à ce phénomène (Hungate ³) dans le cas des pourritures blanches, alors qu'il est plus élevé ailleurs, et tout permet de penser que la sciure, si on ne lui fournit pas d'N, est le siège d'une fixation lente, mais non négligeable. On peut attribuer celle-ci aux *Clostridium* du groupe acéto-butyrique que nous rencontrons dans les horizons inférieurs et, surtout peut-être, à la faune qui, suivant la règle, est abondante dans les régions de teinte sombre. Il ne faut pas négliger non plus

la possibilité d'apports extérieurs, mais ceux-ci paraissent de peu d'importance.

2) Nous avons déjà noté que les dosages de substances humiques montraient de larges variations pour un même produit suivant la date du prélèvement, mais nous ne savons pas s'il s'agit de fluctuations saisonnières ou de différences fondamentales entre échantillons apparemment semblables mais dont les évolutions peuvent avoir été diverses.

En tous cas, le tas de copeaux initialement homogène devient très vite hétérogène: teinte, structure, résistance mécanique varient d'un point à l'autre. Cette hétérogénéité, liée à la présence de populations différentes, est commandée par les conditions écologiques locales: pH élevé au voisinage du sol calcaire, dessiccation rapide en surface, aération réduite et humidité forte en profondeur; mais elle peut aussi dépendre de la nature des espèces ou des associations pionniers, c'est-à-dire, jusqu'à un certain point du hasard, de la proximité d'une ancienne zone pourrie ou d'une planchette par exemple.

3) Dans un premier type évolutif, de loin le plus fréquent, le produit conserve sa texture mais se décolore et s'acidifie - pourriture blanche. Il rappellerait alors quelque peu nos cultures pures de *Trechispora*, mais son peuplement, malgré la dominance des lignivores, reste toujours mixte. Aussi, plus tard, peut-être quand la substance ligneuse est assez dégradée, le produit s'assombrit, sa population se diversifie en même temps que son pH s'élève et ceci correspond à la synergie *Trechispora-Cytosporella* que nous avons décrite *in vitro* ⁴. Une telle évolution correspond aux échantillons C9 à C11 et aujourd'hui la série C ne comporte même plus de région décolorée reconnaissable. Il est probable que D6 correspond lui aussi à une ancienne pourriture blanche en cours de transformation.

4) Le deuxième type de dégradation, beaucoup plus exceptionnel, représenté par le seul D7, se caractérise par une teneur forte en insoluble et une capacité d'échange élevée; il correspond, tout en montrant une évolution plus poussée, à ce que nous avons observé *in vitro* en ensemencant une sciure additionnée de CO_3Ca par un inoculum mixte non lignivore ⁴.

5) Les taux de lignine - c'est-à-dire d'insoluble - et d'humus extractible (acides humiques + acides fulviques) varient en sens

inverses. Dans le cas d'une pourriture blanche (cf. D1, D2, C10) le premier reste constant ou diminue légèrement tandis que les seconds atteignent des valeurs fortes. On remarque en outre que le rapport acides fulviques/acides humiques s'élève (C10, D3) et même que des substances colorées et hydrosolubles se forment dans ces régions et paraissent diffuser dans les couches sous-jacentes; en tout cas la teneur de ces dernières en humus extractible est élevée (C15, D6).

Ainsi les phénomènes que nous enregistrons dans le bois offrent, à première vue de remarquables analogies avec les processus décrits dans les litières et dans les sols tourbeux (cf. p.ex. Frercks et Puffe¹). On sait en effet que, dans ces milieux, une réaction neutre ou alcaline – ou le chaulage – entraîne une forte altération de la structure initiale du produit; les teneurs en N et en insoluble s'élèvent et des acides humiques bruns peuvent se former. Au contraire, dans des conditions dites défavorables (pauvreté en N, pH bas) les débris végétaux restent distincts, leur teinte pâlit, la fraction insoluble diminue, le taux de cellulose augmente. On a même signalé parfois des produits hydrosolubles analogues à l'humus.

Il est très probable que, dans nos copeaux, la prépondérance de ce deuxième type évolutionnel – lié à la présence de lignivores – est due à la pauvreté du bois en N et en C facilement assimilable. Les populations microbiennes banales, ralenties dans leur développement, laissent la place aux formes plus spécialisées, capables de disloquer les liaisons lignine-cellulose-hémicelluloses. Il faut aussi se rappeler que la podzolisation se produit sous les litières résistantes à la décomposition (sous-bois de résineux, landes à Ericacées). Nous ignorons, il est vrai, si, dans ce cas, des lignivores interviennent: leur répartition dans le sol est en effet bien mal connue faute de méthode pratique pour les mettre en évidence. Cependant Warcup⁷ découvrait, dans des sols cultivés, des formes actives dont la présence n'avait pas encore été démontrée.

RESUMÉ

Les auteurs ont étudié la composition chimique (N, substances humiques, lignine) de copeaux de bois blanc exposés à l'air libre depuis 2 à 10 ans, ainsi que leur type de peuplement fongique. En l'absence de lignivore, le pH, les taux de lignine et d'N s'élèvent et il se forme des quantités modé-

rées d'humus à forte capacité d'échange. Dans le cas contraire, on voit apparaître, en abondance, des substances humiques peu condensées et la matière organique subit une évolution rappelant celle du mor.

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BIBLIOGRAPHIE

- 1 Frercks, W. et Puffe, D., Untersuchungen über die Zersetzung der organischen Substanz in Moorböden und Sandmischkulturen mittels der Stoffgruppenanalyse, insbesondere unter dem Einfluss der Kalkung. *Z. Pflanzenernähr. Düng. Bodenk.* **83**, 7-27 (1958).
- 2 Henin, S., Remarques sur l'humus et la matière organique des sols. *Compt. Rend. Acad. Agr. France* **44**, 36-41 (1958).
- 3 Hungate, R. E., Nitrogen content of sound and decayed coniferous woods and its relation to loss of weight during decay. *Botan. Gaz.* **102**, 382-392 (1940).
- 4 Jacquin, F. et Mangelot, F., Populations microbiennes des bois. III. Humification in vitro d'une sciure de hêtre. *Plant and Soil* **11**, 377-391 (1959).
- 5 Kononova, M. M., *Die Humusstoffe des Bodens.* 341 pp. Berlin (1958).
- 6 Mangelot, F., *Recherches méthodiques sur les champignons de certains bois en décomposition.* Thèse Fac. Sci. Nancy. 115 pp. 2 Pl. (1952).
- 7 Warcup, J. H., Studies in Basidiomycetes in soil. *Trans. Brit. Mycol. Soc.* **42**, 45-52 (1959).

ON THE SHAPE OF THE YIELD CURVE

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INTRODUCTION

Prediction of fertilizer requirements of various crops under different soil conditions is one of the main tasks of many research projects in the field of soil fertility. Investigations of the relation between nutrient supply and crop yield are generally based on yield curves. The best known is the one based on the Mitscherlich equation ^{2 3 4 6 10}. Others have shown that a good fitting of experimental data is possible to different yield curves ^{8 11 12 13 14 15 16}.

In the present investigation a study of the yield curves on two soils was undertaken. One of the two soils chosen has a very low amount of available phosphorus and a complete yield curve due to phosphorus fertilization is obtainable, a feature important in a study of the shape of the yield curve.

MATERIALS AND METHODS

Some of the characteristics of the two soils used in the experiment are given in Table 1.

TABLE 1

Some characteristics of experimental soils						
Soil	Texture	CaCO ₃ %	Organic C %	pH	Available P	
					acid extr. ppm	water-resin extr. ppm
Lyngby	loam	0.00	1.36	6.1	20.5	23.0
Troldhede	sand	0.04	2.25	6.6	1.3	3.3

The available phosphorus in the soil was estimated by two methods: by an acid extraction ⁹ and by a water extraction in the presence of an anion exchange resin ¹. pH was determined by means of a glass electrode

in soil paste, organic carbon and CaCO_3 by dry combustion. Nitrogen in plants was determined by the Kjeldahl method.

The experiments were carried out in pots, each pot containing about 20 kg of soil.

The irrigation and care of the crop was carried out by a greenhouse technician according to the standard methods of the laboratory. P^{32} -labelled superphosphate and gypsum were mixed with the soil before placing in the pots. The phosphorus content of the plants was determined by the ammonium-molybdate method in ash obtained by dry ashing. Measurements of radioactivity were performed by a Brüel and Kjaer electronic counter in a liquid counting cell. The activity of the ash solution was measured and compared to activity of superphosphate solution. The superphosphate used in the experiment contained 8% water-soluble P.

Mitscherlich yield curves fitting the data were calculated as described by Eid, Black *et al.*¹⁰. In addition, Putter* adapted a Michaelis equation generally used for enzymatic reactions, for calculation of yield curves, and worked out a procedure for fitting the parameters of the equation to the data. As this procedure is new it seems appropriate to describe it in detail.

The original equation is: $K_m = S(V/v - 1)$, where K_m represents the Michaelis constant, v - rate of the reaction of substrate concentration S , and V - maximum rate of reaction¹⁷.

The equation can be written in the form:

$$v = V - \frac{VK_m}{S + K_m}.$$

Strictly speaking, it can be used in this form only when velocities of reaction are investigated. However, as used in biochemical research, it is frequently applied to final reaction yields. The justification of this practice need not concern us in this context, since we do not claim that crop yield production actually involves a Michaelis mechanism. The Michaelis equation, just like the Mitscherlich equation, is to be regarded merely as an empirically fitted relationship, whose parameters correspond roughly to intuitive and hypothetical concepts like "maximum yield" or "growth rate factor".

In applying the Michaelis equation, we have to take into account the fact that the soil has a nutrient content to begin with. Changing the notation, we have the equation

$$y = A - \frac{Ac}{x + b + c},$$

* Putter, J., Personal communication.

where y is the yield obtained by adding x units of fertilizer to a soil containing an amount of nutrients equivalent to b units of fertilizer. A is the maximum yield obtainable under the given conditions, and c can, if one prefers, be called a "growth-rate factor". Thus, the three parameters correspond intuitively to those of the Mitscherlich equation; in the case of A and b (but not c) the correspondence is complete enough to allow direct numerical comparison of the values obtained by fitting the two equations.

It is convenient to write the equation in the form

$$y = A - \frac{q}{x + r},$$

where $q = Ac$ and $r = b + c$. The least-squares estimates of A , q and r can be obtained as follows. The residual sum of squares can be shown to be $\sum (y_k - \bar{y})^2 - S_1^2/S_2$, where

$$S_1 = \sum y_k/(x_k + r) - \bar{y} \sum 1/(x_k + r);$$

$$S_2 = \sum \frac{1}{(x_k + r)^2} - \frac{1}{n} \left(\sum \frac{1}{x_k + r} \right)^2;$$

n is the number of fertilizer levels x_k , corresponding to yields y_k ; $k = 1, 2, \dots, n$; and $\bar{y} = (x_1 + x_2 + \dots + x_n)/n$. Therefore, the least-squares estimate of r is that value of r which maximizes $f(r) = S_1^2/S_2$. This value is found first, e.g. by a trial-and-error method. The problem then becomes one of ordinary linear regression, and the least-squares estimates of A and q are

$$q = -S_1/S_2 \text{ and } A = \bar{y} + \frac{q}{n} \sum \frac{1}{x_k + r},$$

where the value substituted for r is the one already obtained. The least-squares estimates of b and c are, of course, obtained from this by putting $c = q/A$ and $b = r - c$.

Different amounts of superphosphate were added to the various treatments on the two soils. In addition, other nutrients were applied at two different levels as a heavy and a light basic treatment. In this way, the influence of the various levels of superphosphate was tested at the two levels of general fertility on each soil.

In Tables 2 and 3 the amounts of basic fertilizers and superphosphate at the two different levels are recorded.

$\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ was added to all treatments in such an amount as to supplement the gypsum contained in the highest amount of superphosphate. All treatments were replicated five times.

TABLE 2

Fertilizer	Amounts of basic fertilizers applied to both soils	
	Grams of fertilizer per pot	
	Heavy basic treatment	Light basic treatment
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	15	3.75
KNO_3	10	2.50
NH_4NO_3	3	0.75
$\text{Na}_2\text{B}_4\text{O}_7$	0.1	0.1
$\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$	1.0	1.0
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.5	0.5
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	0.5	0.5

In a laboratory experiment the two soils were mixed with various amounts of superphosphate in order to obtain the same soil-superphosphate relations as in the pots. The samples were brought to the same moisture level as the soils in the pots. After two weeks phosphorus was extracted by the acid method ⁹, and by a water extract ⁵.

EXPERIMENTAL

Oats were sown at end of April 1958 and thinned to 25 plants per pot. The plants were harvested about ten days after the start of earing, in mid July, oven dried and weighed.

Total phosphorus and radioactivity in the green part of the plants were determined. Analysis of total nitrogen in the plants was also performed. The results are given in Table 3.

The yields of the replicates were very uniform, and the *F* value in the analysis of variance of the results was over 200. In both soils there is a very marked effect of phosphorus fertilization on plant yields.

The percentage of phosphorus in plants does not follow the yield curve, but reaches a minimum at low concentrations, which does not appear in the yield curve. The nitrogen percentage drops with increase of yield, especially in the light basic treatment.

In order to study the functional relationship between the amount of nutrient supplied and plant yield, yield curves were calculated.

An attempt was made to fit a yield curve to the data in three ways: 1) The parameters of the Mitscherlich equation best fitting

TABLE 3

Yield of oats and phosphorus percentages in dry matter, average per treatment									
Soil	Grams supplied		Heavy basic treatment			Light basic treatment			
			Yield of dry matter, grams	% of P in plants		Yield of dry matter, grams	% of P in plants		% of N in plants, total
	Super-phosphate	P		Total	Derived from super-phosphate (radioactive)		Total	Derived from super-phosphate (radioactive)	
Trolldhede	0	0	4.6	0.12	—	5.3	0.20	—	2.6
	2.5	0.2	6.6	0.14	0.04	9.3	0.20	0.07	2.5
	5	0.4	10.6	0.14	0.07	11.9	0.20	0.09	2.3
	10	0.8	16.7	0.14	0.09	17.6	0.19	0.11	2.2
	20	1.6	30.9	0.13	0.10	34.6	0.16	0.12	2.0
	40	3.2	61.7	0.14	0.11	58.0	0.14	0.12	1.4
	80	6.4	82.0	0.16	0.14	74.1	0.15	0.14	1.1
	160	12.8	96.8	0.20	0.18	82.6	0.19	0.18	1.1
Lyngby	320	25.6	104.0	0.25	0.23	87.0	0.25	0.24	1.0
	0	0	31.0	0.20	—	39.3	0.21	—	1.6
	5	0.4	40.2	0.19	0.02	46.9	0.19	0.02	1.4
	10	0.8	49.7	0.18	0.04	52.4	0.17	0.03	1.2
	20	1.6	53.2	0.19	0.08	58.5	0.17	0.06	1.1
	40	3.2	67.7	0.20	0.11	66.1	0.20	0.10	1.0
	80	6.4	74.1	0.23	0.16	69.8	0.24	0.15	1.0
	160	12.8	83.6	0.33	0.24	73.0	0.30	0.22	1.0

the yield results were sought¹⁰. The resulting equations and curves are given in Fig. 1. 2) The data for Trolldhede soil suggest that the yield curves have an S-shape and, accordingly, free-hand curves connecting the experimental points were drawn (Fig. 1). The

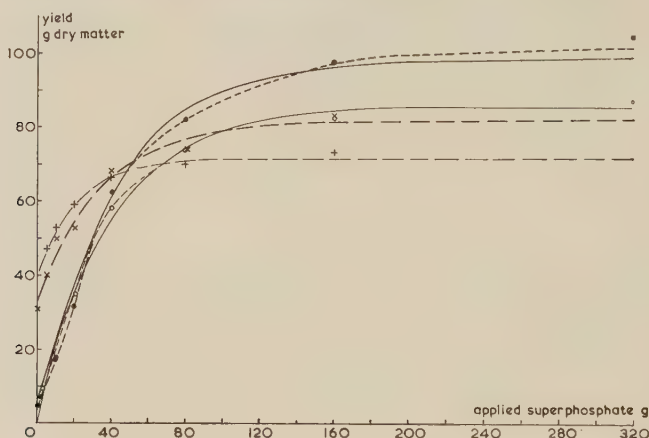


Fig. 1. Yield curves derived from data in Table 3, according to the logarithmic equation and (for Trolldhede soil only) free-hand curves.

- - - - ● Trolldhede heavy basic (free hand)
- - - - ○ „ light „ („ „)
- - - - ● „ heavy „ $\log(98.73 - y) = \log 98.73 - 0.011(x - 0.83)$
- - - - ○ „ light „ $\log(85.94 - y) = \log 85.94 - 0.011(x + 1.51)$
- × - - - × Lyngby, heavy „ $\log(82.01 - y) = \log 82.01 - 0.013(x + 17.80)$
- + - - - + „ light „ $\log(71.83 - y) = \log 71.83 - 0.019(x + 18.27)$

curves for the Lyngby soil start much higher on the y-axis and the S-shape is not obvious. 3) Furthermore, the Michaelis equation, an equation used for enzymatic reaction, was fitted to the experimental data (Fig. 2). Judging the fitting of this last equation to the experimental data, by the sum of squares of deviations of the data from the curve, it appears that it is as good as the logarithmic equation.

The intercept on the x-axis between the values for $y = 0$ and the origin of the coordinate system is, in many cases, used as an indicator of the amount of available nutrient present in the soil. (For example, it is represented in the Mitcherlich equation by the value for b). Therefore it seems that the evaluation of this intercept is of critical importance. This may be done as proposed by Steenbjerg¹⁴, by relating the dry-matter yield of plants to the

phosphorus absorbed by them. The resulting curve is based on the experimental data, except for one additional point on the curve which is fixed, namely that when the yield is zero, the amount of absorbed phosphorus is also zero. (The total amount of phosphorus supplied by the seeds to the whole plant is negligible, *i.e.* approximately 2.5 mg).

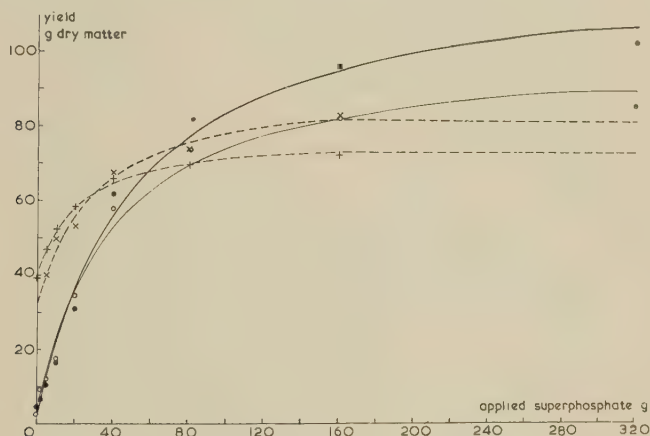


Fig. 2. Yield curves derived from data in Table 3 according to Michaelis equation.

● — ●	Trolldhede, heavy basic:	$y = 125.5 - \frac{125.5 \times 19.9}{x + 0.1 + 19.9}$
○ — ○	Trolldhede, light basic:	$y = 102.0 - \frac{102.0 \times 15.1}{x + 0.4 + 15.1}$
× — ×	Lyngby, heavy basic:	$y = 92.6 - \frac{92.6 \times 4.0}{x + 11.5 + 4.0}$
+ — +	Lyngby, light basic:	$y = 78.7 - \frac{78.7 \times 2.2}{x + 3.9 + 2.2}$

By free-hand connection of the zero point and the experimental points (Fig. 3), curves are obtained, which for the Trolldhede soil (a very phosphorus-deficient soil) are similar to the curves in Fig. 1, where the dry-matter yield is presented as function of the phosphorus applied.

The curves for the Lyngby soil in Fig. 3 show a very definite sigmoid shape. In cases where the sigmoid shape is not obvious from the usual yield curve, its existence may be shown by this way of presentation.

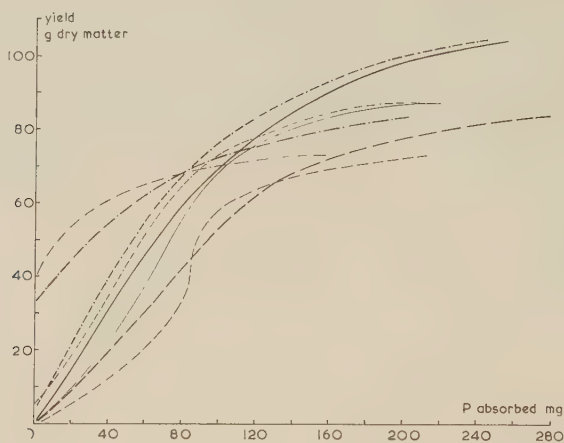


Fig. 3. P absorbed by plants versus yield of dry matter.

————— Trolldhede, heavy basic, Total P
 - - - - - " " " Superphosphate P
 ———— " light basic, Total P
 - - - - - " " " Superphosphate P
 ————— Lyngby, heavy basic, Total P
 - - - - - " " " Superphosphate P
 ———— Lyngby, light basic, Total P
 - - - - - " " " Superphosphate P

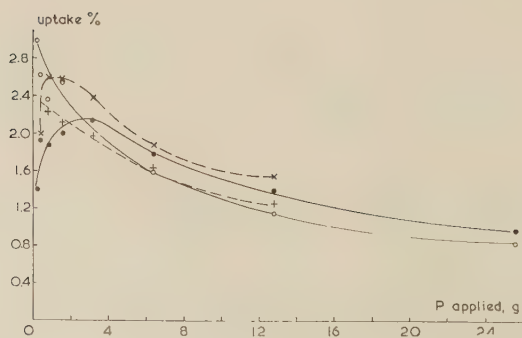


Fig. 4. Percentage of phosphorus uptake by plants from superphosphate.

● ———— ● Trolldhede, heavy basic
 ○ ———— ○ Trolldhede, light basic
 × ———— × Lyngby, heavy basic
 + ———— + Lyngby, light basic

Percentage of phosphorus absorbed by plants from the superphosphate applied was calculated (Fig. 4). For both soils the pattern of absorption at the heavy basic treatment is different from the one with the light basic treatment. In the light basic treatment there is a gradual decrease of percentage of absorption with increased supply of superphosphate, while with the heavy basic treatments a maximum is found.

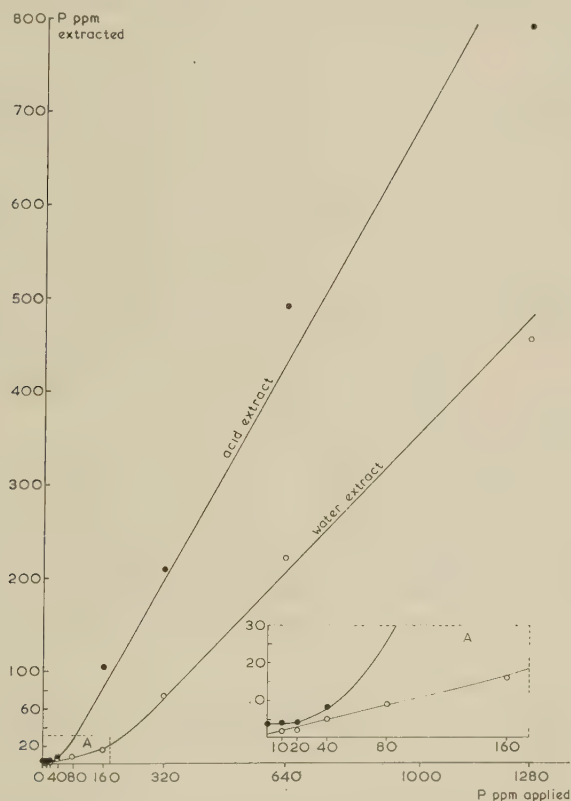


Fig. 5. Relation between applied and extracted phosphorus in Trolldhede soil.

In a laboratory experiment where increasing quantities of superphosphate were mixed with the two soils and subsequently extracted, it was found that relatively less phosphorus was recovered from the lower applications than from the higher ones (Figs 5 and 6).

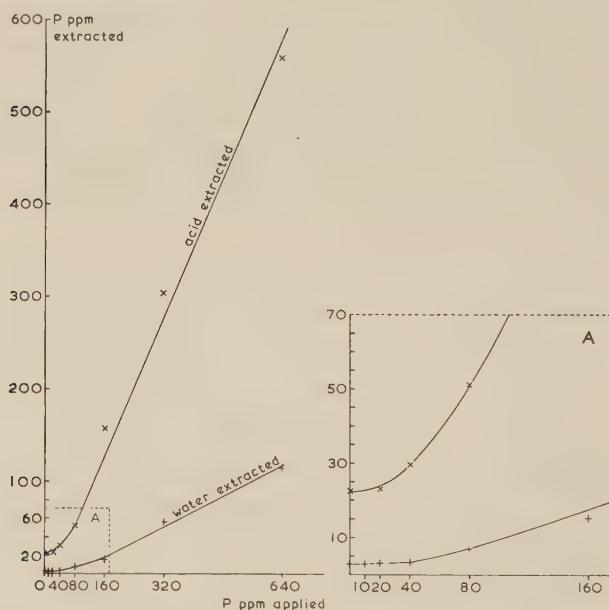


Fig. 6. Relation between applied and extracted phosphorus in Lyngby soil.

DISCUSSION

The experiment showed that on very deficient soils, where a nearly complete yield curve is obtainable, the data could best be fitted to a sigmoid curve. The first increments of phosphorus fertilizer did not increase the yield as much as would be expected from a Mitscherlich or Michaelis curve. Steenbjerg¹³ showed that even on less deficient soils an indication of the sigmoid shape of the yield curve could be obtained, and calculations from this experiment verified that finding. Also, the known "Steenbjerg effect" is a possible indication of the sigmoidal shape of the yield curve. As pointed out by Steenbjerg, small additions of a deficient nutrient at first depress the percentage of the nutrient absorbed by the plants, and only larger additions result in an increase in the nutrient level in the plants. This same effect may be seen in the results of phosphorus determinations in plants in this experiment.

The laboratory experiments showed that relatively much smaller

amounts of phosphorus are extractable when small amounts are added, than when larger quantities are applied to the soil.

All these findings indicate a strong fixation of phosphorus by soil when added in small amounts. Only after a certain quantity is added and the fixing power of the soil is saturated, is the added phosphorus freely available to plants. In consequence, the first additions of nutrient to a deficient soil induce only relatively small yield increases and only the later ones show increases which produce a yield curve fitting some logarithmic or exponential equation.

It is interesting to note that the plants react differently to the same amount of phosphorus at a lower level of application of other fertilizers, than at a higher one. The basic treatment gives higher yields at lower phosphorus applications, but lower yields at higher application, when compared to the heavy basic treatment. No explanation is offered for this fact. However, it indicates that interactions between nutrients are complicated, and simple multiplication of effects may be erroneous.

SUMMARY

A greenhouse experiment with phosphorus fertilization on two soils, one of them very deficient in nutrients, was performed with oats. Dry-matter yields, total-phosphorus percentage and superphosphate-phosphorus percentage (by means of radioactivity measurements) in plants were determined. Yield curves drawn by free hand and calculated according to the Mitscherlich and Michaelis equations were compared. It was concluded that the experimental data indicate a sigmoid-shaped yield curve.

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REFERENCES

- 1 Amer, F. *et al.*, Characterization of soil phosphorus by anion exchange resin adsorption and P^{32} -equilibration. *Plant and Soil* **6**, 391-408 (1955).

- 2 Balba, A. M. and Bray, R. H., New fields for the application of the Mitscherlich equation: 1. A quantitative measure for the relative effectiveness of nutrients. *Soil Science* **81**, 497-502 (1956).
- 3 Balba, A. M. and Bray, R. H., New fields for the application of the Mitscherlich equation: 2. Contribution of soil nutrient forms to plant nutrient uptake. *Soil Science* **83**, 131-139 (1957).
- 4 Balba, A. M. and Haley, L. E., Comparison of results obtained by the Balba-Bray equation and radioactive techniques for the determination of nutrient uptake by plants from different nutrients forms. *Soil Science* **82**, 365-368 (1956).
- 5 Bingham, F. T., Soil test for phosphate. *California Agr.* **3**, 11 (1949).
- 6 Black, C. A. and Scott, C. O., Fertilizer evaluation: 1. Fundamental principles. *Soil Sci. Soc. Am. Proc.* **20**, 176-179 (1956).
- 7 Black, C. A., Webb, J. R. and Kempthorne, O., Fertilizer evaluation: 3. Availability coefficient of water-soluble and citrate-soluble phosphorus in acidulated phosphate fertilizers. *Soil Sci. Soc. Am. Proc.* **20**, 186-189 (1956).
- 8 Bondorff, K. A., The evaluation of soil analysis. *Trans. Intern. Soc. Soil. Sci. Dublin 1952* **1**, 290-295 (1953).
- 9 Bondorff, K. A. og Steenbjerg, F., Studier over jordens fosforsyreindhold. 1. Yord fosforsyreens opløselighed. *Tidsskr. Planteavl* **38**, 273-308 (1932).
- 10 Eid, M. T., Black, C. A., Kempthorne, O. and Zoellner, J. A., Significance of soil organic phosphorus to plant growth. *Iowa State Coll. Agr. Expt. Sta. Research Bull.* **406** (1954).
- 11 Paauw, F. Van der, Calibration of soil test methods for the determination of phosphate and potash status. *Plant and Soil* **8**, 105-125 (1956).
- 12 Paauw, F. Van der, Evaluation of methods of soil testing by means of field experiments. *Trans. Intern. Soc. Soil. Sci. Dublin 1952* **1**, 207-221 (1952).
- 13 Steenbjerg, F., A theory on the placement of fertilizers. *Roy. Vet. Agr. Coll. Copenhagen Yearbook* **1957**, 1-30 (1957).
- 14 Steenbjerg, F., Manuring, plant production and the chemical composition of the plant. *Plant and Soil* **5**, 226-242 (1954).
- 15 Steenbjerg, F., Verification of chemical soil and plant analysis. General considerations. *Trans. Intern. Soc. Soil Sci. Dublin 1951* **1**, 309-322 (1952).
- 16 Steenbjerg, F., Yield curves and chemical plant analysis. *Plant and Soil* **3**, 97-109 (1951).
- 17 Sumner, Y. B. and Somers, G. F., *Chemistry and Methods of enzymes*, pp. 415 Academic Press, N.Y. (1947).
- 18 White, R. F., Kempthorne, O., Black, C. A. and Webb, J. R., Fertilizer evaluation: 2. Estimation of availability coefficients. *Soil Sci. Soc. Am. Proc.* **20**, 179-186 (1956).

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